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Three Ascending Spinal Pathways in the Dorsal Part of the Lateral Funiculus

By

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Abstract

LUNDBERG, A. and O OSCARSSON. *Three ascending spinal pathways in the dorsal part of the lateral funiculus.* Acta physiol. scand. 1961 *51* 1—16. — Three pathways with axons ascending in the dorsal part of the lateral funicle have been identified as not belonging to the dorsal spino-cerebellar-tract because the axons could be activated on stimulation of the lateral funicle in L5 below the caudal level of Clarke column but not antidromically from the anterior cerebellar lobe cortex. One of these pathways is located medial to the dorsal spino-cerebellar tract and is activated exclusively by tactile stimuli from small skin fields.

The activity in ascending pathways of the dorsal part of the lateral funicle (Flechsig's fasciculus) was analyzed by mass discharge and unit recording (LAPORTE, LUNDBERG and OSCARSSON 1956 a, b, LAPORTE and LUNDBERG 1956) In a previous report units belonging to the dorsal spino-cerebellar tract (DSCT) were identified by antidromic stimulation from the anterior cerebellum (LUNDBERG and OSCARSSON 1960) The present paper deals with the identification of three ascending pathways also with axons in the dorsal part of the lateral funiculus, but not belonging to the DSCT Identification was achieved not only because these axons could not be activated antidromically from the cerebellum but also from the finding that they were activated by a stimulus applied to the lateral funicle below the caudal end of Clarke's column (REXED 1954) Evidence that there are other ascending pathways in Flechsig's fasciculus than the DSCT has been presented by BRODAL and REXED 1953, GRUNDFEST and CARTER 1954 WALL (1960) ECCLES, ECCLES and LUNDBERG (1960) A preliminary report of these results has been published (LUNDBERG and OSCARSSON 1959)

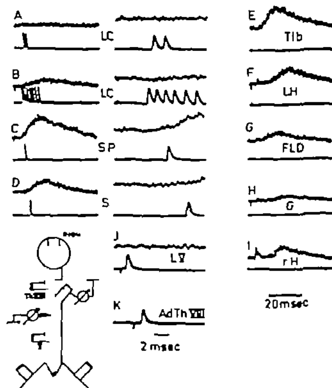


Fig. 1. Microelectrode recording (lower traces) from an axon of the tactile tract and mass discharge recording (upper traces) from the contralateral spinal half. The left and right traces in records A—D were taken simultaneously at different speeds. E—I were taken at the lower speed and J and K at the faster. Records B—I show the effect of supramaximal stimulation of the nerve indicated in each record. Stimulation in A was supraliminal. Abbreviations: L. C, lateral cutaneous; S P, superficial peroneal; S, sural; Tib, tibial; L. H, hamstring; FLD, flexor digitorum longus; G, gastrocnemius-soleus (all left nerves); rH, right hamstring. Stimulation of the fibre by stimulus applied in L5 and Th8 is shown in J and K.

Distance from site of microelectrode recording to stimulating electrode in L5 4.7 cm and to stimulating electrode in Th8 7.3 cm.

Methods

The experiments were performed at the same time as the investigations reported in a previous paper on the dorsal spino-cerebellar tract (LUNDBERG and OSCARSSON 1960) and the methods were identical with those described in this paper. The experiments were made on unanaesthetized cats decerebrated by intercollicular section. Microelectrode recording was made from axons in the dorsal part of the left lateral funicle in L1. For control the mass discharge was recorded in the lower thoracic region from the contralateral right spinal half or from the dissected right ventral quadrant.

Results

1. *The tactile tract*

a) *Unit recording*

These neurones constitute an easy identifiable and homogenous group. The axons conduct at 100—60 m/sec. At electrical stimulation they are activated only from cutaneous nerves and not by muscle afferents. This is illustrated in Fig. 1 for a unit receiving its main activation from the lateral cutaneous nerve (which penetrates the lower part of biceps and innervates the lateral part of the leg below the knee). Record A shows the effect of stimulation of this nerve

at threshold strength, which does not give rise to a mass discharge in the contralateral spinal half (upper trace) at stronger stimulation in B there is a train of 8 impulses. In all of these neurones the latency of the first spike in response to a volley in the nerve supplying the main excitation was so brief that transmission must be monosynaptic. Record C and D Fig 1 show that there was some subsidiary excitation from the superficial peroneal (C) and the sural (D) nerve, with one spike on supramaximal stimulation of each of them. There was no effect on supramaximal stimulation of any of the ipsilateral muscle nerves F—H or from the mixed tibial nerve (E) Stimulation of contralateral nerves did not in any case evoke excitation (I Fig 1) These axons could not be antidromically activated from the cerebellum but were stimulated by a shock applied to the lateral funicle in L5 (record J Fig 1) which is below the caudal level of Clarke's column (REXED 1954)

When tested for adequate activation these units were found to respond extremely effectively (often at frequencies above 500/sec) to light touch, all of them were in fact activated by blowing of hairs. It was not possible to observe any increased activation on pressure or pinching of the skin and thereby these units differ radically from the two subgroups of the DSCT (LUNDBERG and OSCARSSON 1960) and also from units described in section 2 of this paper of which many are activated by tactile stimuli. The receptive fields were usually quite small, with many of the units activated from the toes, excitation was provided from a skin area of about 10 mm more proximally on the limb larger receptive fields were found and on the proximal part of the thigh or the lower trunk they could be as large as 20 cm In this connection it should be considered that receptive fields of primary tactile afferent diminish in a distal direction (YAMAMOTO, SUGIHARA and KURU 1956, LINDBLOM 1958 cf., however WALL, 1960) and that similar findings have been made on units in Flechsig's fasciculus (YAMAMOTO and MIYAJIMA 1959) and also on cells in the cats sensory cortex (MOUNTCASTLE 1957)

These tactile units have a resting discharge (like the neurones of all other pathways we have investigated) against which inhibition easily can be disclosed. However there was never any inhibition on touch, pressure and pinching of the skin of the hindlimbs and the trunk. Identical findings were made by WALL (1960) on tactile cells in the dorsal horn of the lower lumbar region. In summary the organization of connections to the tactile tract is rather simple, it forwards a modality specific message which is highly spatially discriminative.

b) *Contribution to mass discharge and location of pathway in Flechsig fasciculus*

This investigation of non-DSCT pathways in the dorsal part of the lateral funicle, was in part based on a comparison of evoked cerebellar potentials and the discharges in dissected Flechsig's fasciculus (LAPORTE *et al.* 1956 a, LUNDBERG and OSCARSSON 1960) On stimulation of skin nerves (cf. Fig 5 B and D LUNDBERG and OSCARSSON 1960) no correspondence was found in the

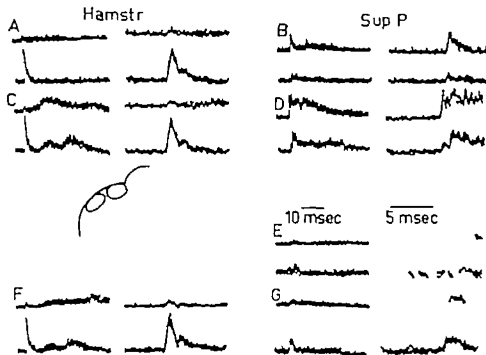


Fig. 4 Simultaneous recording in the lower thoracic region from two dissected filaments of Flechsig' fasciculus in spinal cat. One medial and one lateral fasciculus was dissected as shown in the drawing and in each record the upper trace is from the medial and the lower from the lateral fasciculus. The ipsilateral hamstring (Hamstr.) nerve was stimulated in A, C and F in A at a strength of 2 times threshold and in C and F at 30 times threshold. Stimulation of the ipsilateral superficial peroneal (Sup. P.) nerve in B, D, E and G in B and E at strength of 1 05 and in D and G at 20 times threshold. Records E, F and G were taken after section of the dorsal part of the lateral funicle in L3.

small early cutaneous discharge in the lateral fasciculus have disappeared It should be observed that the main cutaneous discharge in the lateral fasciculus in D and the corresponding remaining discharge in G after the lesion have their onset 0 8 msec later than the early cutaneous discharge. This discharge in the lateral fasciculus is probably mainly due to activity in DSCT neurones monosynaptically activated by cutaneous afferents (LUNDBERG and OSCARSSON 1960) but a contribution from the pathway described in the next section cannot be excluded (cf. below) A longer latency would be expected in DSCT units because of the relatively slow conduction in the dorsal column in which the cutaneous afferents ascend to reach Clarke's column. The difference of 0 8 msec in the experiment of Fig 4 was exceptionally long, the usual difference was 0.3 msec

The pathway described in the next section is also monosynaptically activated by cutaneous afferents and should contribute to the non-DSCT cuta

neous discharge in Flechsig's fascicle. Most of these axons were located medially but we cannot from our material exclude the possibility that they are more widely distributed in Flechsig's fascicle than those of the tactile tract. These units were not common, only 12 were found against more than 50 of the tactile tract. Presumably the early cutaneous discharge recorded medially is due largely to activity in the tactile tract.

The recordings in Fig. 5 are of special interest in connection with the early work on pathway in Flechsig fasciculus and may serve to explain some findings which have puzzled us. In their work on the DSCT GRUNDFEST and CAMPBELL (1942) recorded discharges evoked by volleys in group I muscle afferents, but did not then observe any effects when stimulating cutaneous nerves. On the other hand when recording from the dissected Flechsig fasciculus the characteristic cutaneous discharge is always found (LAPORTE et al., 1956 a). Presumably GRUNDFEST and CAMPBELL (1942) recorded laterally in the true location of the DSCT where the cutaneous discharge is not so conspicuous and may have escaped notice. LLOYD and MAC INTYRE (1950) based their analysis of the DSCT on GRUNDFEST's and CAMPBELL's finding that no discharge was evoked from cutaneous afferents. They stimulated the dorsal roots and assumed that discharge recorded in the ascending pathway was evoked only by muscle afferents. The discharge recorded by them was different from that found by GRUNDFEST and CAMPBELL and from discharge now known to be due to activity in the DSCT (records A and B, Fig 5) but it is very similar to the discharge in record C, Fig 5. Their recording position probably was medial and the discharge found by them probably due to activity in the tactile tract. It should also be mentioned that GRUNDFEST and CARTER (1954) have found cutaneous discharge in the lateral funicle which they ascribe to activity in a spino-olivary pathway.

c) *Is transmission in the tactile tract supraspinally controlled?*

For the understanding of the function of any pathway it is important to learn about the supraspinal controlling systems which may influence transmission. We have not made an exhaustive study of the control of the tactile tract but have investigated if any of the two supraspinal control systems analysed by HOLMQVIST, LUNDBERG and OSCARSSON (1960 a, b) has an effect. One of these systems takes origin in the anterior cerebellum, relays in the brain stem and activates monosynaptically the cells of a ventral pathway (HOLMQVIST, LUNDBERG and OSCARSSON 1960 b). This system does not connect with the tactile pathway as evidenced by the finding that the resting discharge in these units is not influenced by repetitive stimulation of the anterior cerebellum. Neither was there any effect on transmission to this pathway tested with the single volley technique and with adequate activation. Another supraspinal control system inhibits effectively the interneurones of the flexion reflex pathway and the interneurones mediating effects to a number of ascending pathways influenced by the flexion reflex afferents (HOLMQVIST and LUNDBERG 1959, HOLMQVIST, LUNDBERG and OSCARSSON 1960 a). This control system can be activated by stimulation of the dorsal half of either lateral funicle. This was tried with 15 of the neurones of the tactile tract both with

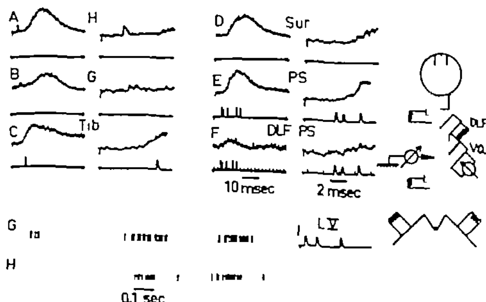


Fig. 5. As in Fig. 1 microelectrode recording from axon of the tactile tract with simultaneous recording of mass discharge in the contralateral dissected ventral quadrant. Stimulation of various hindlimb nerves in A—F. Abbreviations different from those in Fig. 1 are: H, left hamstring, Sur. sural. PS, *superficial peroneal*. In F the *superficial* peroneal nerve was stimulated during repetitive stimulation of the dissected dorsal half of the right lateral funicle (DLF). Observe that there is no effect on the unit discharge while the mass discharge in the contralateral spinal half is strongly depressed. The receptive skin field was on the dorsum of the two lateral toes. I. G and H light touch was applied three times, in G before and in H during repetitive stimulation of the contralateral DLF. Record I shows activation of axon by stimulus applied to the lateral funicle in L5.

the electrically and adequately evoked discharge as is illustrated in Fig. 5. Record E shows that this neurone was activated from the superficial peroneal nerve and there was also one spike on stimulation of the tibial nerve. In record F the superficial peroneal nerve was stimulated during tetanic stimulation of the dissected dorsal half of the right lateral funicle (DLF) but the discharge is unchanged. The upper trace in F shows that transmission of the mass discharge to the dissected right ventral quadrant was very much depressed and this shows that the descending control operated (cf. Holmqvist *et al.* 1960 a). The receptive skin field for this unit was on the dorsal side of the lateral toes and in both G and H light touch was applied to this toe 3 times, in G before and in H during tetanic stimulation of the right DLF. There was no significant change in the effectiveness of the adequate stimulation. However in 7 of the 15 investigated neurones stimulation of the contralateral DLF had a slight inhibitory effect. This effect was probably due to removal of late polysynaptic components. Our interpretation of the finding is that transmission to the tactile tract escapes this control system.

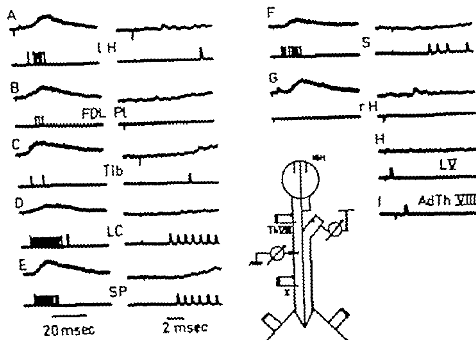


Fig. 6. As in Fig. 1 but recording from an axon activated by cutaneous and by high threshold muscle afferents. Records A—G show the effect of supramaximal stimulation of the nerv. indicated in each record Additional abbreviation from Fig 1 are FDL + Pl, Flexor digitorum longus and plantaris. In record H and I the axon was activated by a stimulus applied as indicated. Distance from site of microelectrode recording to stimulating electrode in L5 4.7 cm and to stimulating electrode in Th8 7.3 cm.

2. *A pathway activated by the flexion reflex afferents*

This is one of the many pathways influenced by the flexion reflex afferents (cf. HOLMQVIST *et al.* 1960 a) It seems to be a twin pathway to one of the DSCT subgroups and is likewise excited by cutaneous and by high threshold muscle afferents from a very wide ipsilateral receptive field as is illustrated in Fig 6. Activation of the fibre by a weak stimulus applied to the lateral *fascicle to L5 is shown in record H. Records A and B show that there was ex-*citation from muscle nerves but this unit received its main activation from cutaneous nerves. Single volleys from the lateral cutaneous (D) superficial peroneal (E) and sural (F) nerves evoked high frequency trains of impulses as contrasted to the more restricted fields of supply to the tactile units. The short latencies of the first spike in D E and F demonstrate monosynaptic action, and all except one of these units was monosynaptically excited from at least one of these cutaneous nerves. However additional polysynaptic cuta-

neous effects were indicated by longer latencies. Also with respect to their mode of adequate activation these units resembled the similar DSCT units. Most of them were activated by tactile stimuli from a restricted skin field which could be as small as few cm but sometimes comprised very much larger areas. All the units activated by tactile stimuli received additional excitation on pressure and pinching and the receptive fields for these effects were larger. A few of the units could only be activated by pressure and pinching. On the basis of the adequate effect it was very easy to distinguish these units from those of the tactile tract. The conduction velocity of the axons of this pathway ranged from 100 to 40 m/sec.

The effect of stimulation of the anterior cerebellum was tested on all these units and in no case was there any significant effect on the resting discharge or on transmission from the hindlimb nerves. Stimulation of the dorsal half of the lateral funicle in order to activate the inhibitory control of interneurones mediating the flexor reflex actions to various pathways (HOLMQVIST *et al.* 1960 a) has been tried extensively with the similar DSCT units but only with few units of the present group. With the DSCT units it has been found that in the majority of the units transmission of the effect from skin (tested adequately and by electrical stimulation of various skin nerves) largely escapes the inhibitory control whereas excitation from high threshold muscle afferents is very effectively suppressed (LUNDBERG and OSCARSSON, unpublished). Similar findings were made with the two non DSCT units tested in this respect, in one the skin effect was unchanged in the other unit there was some reduction of the discharge evoked from skin as well. In addition it has been found with intracellular recording from two cells of this pathway that the EPSP evoked by volleys in high threshold muscle afferents was completely removed by repetitive stimulation of the dorsal half of the lateral funicle on the other side (LUNDBERG and VOORHOEVE, unpublished). The two pathways are probably similar also in this respect. When the supraspinal control operates they are apparently converted to exteroceptive pathways. The reason why the effect from skin to a large extent escapes the control is probably that it depends mainly on monosynaptic connections. However we have no knowledge of the relative importance of mono- and polysynaptic connections to these pathways. Alternatively it would have to be assumed that the supraspinal control selects at an interneuronal level.

It is not possible to ascertain the contribution of this pathway to the mass discharge in Flechsig's fasciculus, as has already been discussed for the cutaneous effects in section 1. The activity evoked on stimulation of high threshold muscle afferents should contribute to the late component of the mass discharge. I. Fig. 2 (A and B, left traces) it can be observed that this component is reduced by 50 per cent after the lesion in the lateral funicle in L5. However it cannot be assumed that this reduction is due entirely to the interruption of the present pathway because cells of Clarke's column could receive synaptic action from collaterals of propriospinal axons ascending in the dorsal part of the lateral funicle (SZENTÁGOTHAI and ALBERT 1955, SZENTÁGOTHAI, personal

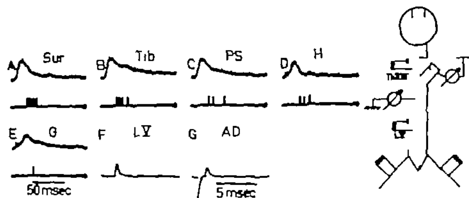


Fig. 7 As in Fig. 6 but recording from an axon activated by cutaneous and high threshold muscle afferents after long latency. Distance from site of microelectrode recording to stimulating electrode in L5 5.5 cm and to stimulating electrode in Th8 6.8 cm.

communication). On the other hand it is not even possible to conclude that the remaining mass discharge in B, Fig. 2, is due entirely to activity in DSCT because non-DSCT axons (of this and the next section) activated by hindlimb afferents may arise rostral to L5.

3. *Neurones activated by the flexion reflex afferents after a long central delay*

In our previous work these units have not been recognized as a separate group but have been classified with the other units activated by the flexion reflex afferents. Their axons conduct at 110—70 m/sec. They are distinguished not only by the longer latencies of the effects evoked from hindlimb nerves but also by being strongly influenced from the anterior cerebellum. Usually they were activated from all ipsilateral hindlimb nerves tested and after a latency of 10—30 msec (Fig. 7 A—E). Often there was only 2—4 spikes but in more excitable preparations trains of 20—30 spikes were observed. The latency of the discharge is so long that it could be descending, but since the same response also was found in spinal animals it is concluded that we are dealing with an ascending pathway. Many of these units received excitation also from contralateral nerves although in the dorsally located ones always less than from the ipsilateral side. This is an exception to the rule that all ascending pathways with axons in the dorsal part of the lateral funicle have an ipsilateral receptive field (OSCARSSON 1958, HOLMQVIST *et al.* 1960 a). Axons of this type are found also more ventrally in the cord and in these units the contralateral effects were more pronounced. Like the other two groups described in this paper these units were identified as not belonging to the DSCT because the axons could be activated by a stimulus applied to the lateral funicle in L5

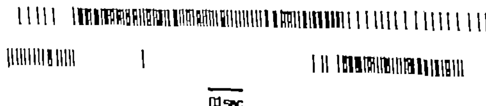


Fig 8. Microelectrode recording from an axon of the type illustrated in Fig 7. Upper record shows excitation during repetitive stimulation (black line) of the ipsilateral vermis of the anterior cerebellum. Lower record shows inhibition during stimulation of the ipsilateral intermediate cortex. The frequency of stimulation was 200/sec.

The effect from the anterior cerebellum was one of the criteria required to classify these units, but there was in fact cerebellar effects on all the units responding to stimulation of peripheral nerves as shown in Fig 7. The effects were excitatory and inhibitory and in some cases it was possible to find cerebellar areas giving only one of these effects. For example, in Fig 8 excitation (upper record) was provided from the left hemivermis and the inhibitory effect from the left intermediate cortex. It was however not possible to denote any area as preponderantly excitatory or inhibitory for these units, since there was a great variability in different units. The most common response was in fact one of mixed excitation and inhibition from the whole width of the anterior cerebellum. At the onset of repetitive stimulation there was an excitatory burst followed by inhibition and on cessation of stimulation a longlasting excitatory rebound occurred. Strong cerebellar effects have also been found on a ventral pathway but in this case it was possible to denote separate excitatory and inhibitory longitudinal zones on the anterior cerebellum (HOLMQVIST *et al.* 1960 b). The possibility should therefore be considered that the effects described here are exerted by another supraspinal control system from the anterior cerebellum. Further experiments are obviously needed to investigate this problem. The cerebellar effects, described, may be of interest to account for some findings by HAGBARTH and FEX (1959). They recorded from axons in Flechsig's fasciculus and observed strong effects in many ascending neurones on stimulation of the anterior cerebellum. We have not observed any marked effects on cerebellar stimulation on any of the 5 DSCT subgroups (LUNDBERG and OSCARSSON unpublished) or on the pathways described in the first 2 sections of this paper. It is possible that the units of HAGBARTH and FEX (1959) belonged to the pathway described in this section. Alternatively they could belong to the ventral pathway described by OSCARSSON (1958); this pathway is also strongly influenced from the anterior cerebellum (HOLMQVIST *et al.* 1960). In the lateral part of Flechsig's fasciculus axons of this pathway are sometimes found at a depth of less than 1 mm below the surface.

At descending stimulation of the contralateral dorsal half of lateral funicle there was very effective suppression of transmission to these units. This could occur without any effect on the resting discharge, which however sometimes was inhibited but more often facilitated.

These units were found frequently and predominantly laterally in Flechsig's fascicle intermingled with the DSCT axons. Presumably they must contribute to the late mass discharge in Flechsig's fascicle. Sometimes this discharge did consist of two components as in record C, Fig. 4. The late component in the left record, with an onset 17 msec after the group I evoked discharge, could be due to activity in the units described in this section. This suggestion is not necessarily contradicted by the fact that the late component of the mass discharge is not abolished (record F, Fig. 4) after transection of the dorsal part of the lateral funicle in L5. Although not being part of DSCT some of these axons may arise rostral to L5. An additional possibility is that these axons have more ventral location in the lower lumbar region and that some were spared by the lesion. On stimulation of the lateral funicle in L3 it was noted that many of these axons had higher threshold than those of the other two pathways of this paper.

Discussion

Of the three non-DSCT pathways described the tactile tract deserves the greatest interest at present. These neurones are activated exclusively by light touch, and there was no additional activation on pressure and pinching as has been found with two subgroups of DSCT neurones (LUNDBERG and OSCARSSON 1960) and with the pathway described in section 2 of this paper. The very small receptive fields of the tactile tract cells makes this pathway highly spatially discriminative. The axons of the tactile tract are located in the most dorsomedial part of the lateral funicle in almost complete anatomical separation from the more laterally located dorsal spino-cerebellar tract. This may be due to the difference in segmental levels from which these pathways arise. It is known that within the dorsal spino-cerebellar tract fibres arising from higher levels of the cord are accumulated more ventrally in the tract (YOSS 1952, VACHANANDA 1959). The separate location of the DSCT and the tactile tract offer technical possibilities to differentiate between the functional significance of these pathways which may be important since a considerable number of dorsal spino-cerebellar tract neurones can be activated by tactile stimuli (LUNDBERG and OSCARSSON 1960).

Presumably the cells of origin of the tactile tract are among those intracellularly recorded from by ECCLES *et al.* (1960). In their investigation cutaneous tract cells with axons ascending in the ipsilateral dorsolateral funicle were found in the dorsal horn of the lower lumbar segment at a depth of 1.6–2.0 mm from the cord dorsum, which would be in the 4th and 5th layer of REXED (1952, 1954). All these cells received monosynaptic excitation from low threshold cutaneous afferents but some were excited by high threshold muscle afferents as well and are probably the cells of origin of the second

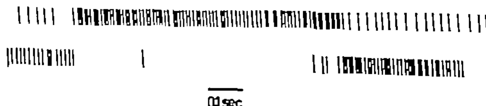


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1) A pathway monosynaptically excited by the lowest threshold cutaneous afferents. These neurones respond adequately to light touch from very restricted receptive fields but do not receive additional activation on pressure and pinching of the skin. The axons of this pathway are located in the mediodorsal part of the lateral funiculus in almost complete anatomical separation from the more lateral dorsal spino-cerebellar tract. There was little or no evidence of a supraspinal control of this pathway

2) A pathway activated by ipsilateral cutaneous and high threshold muscle afferents. These neurones resemble closely one of the subdivisions of the dorsal spino-cerebellar tract. Most of them are activated by tactile stimuli from receptive fields which for some neurones are relatively restricted but for others large. Additional activation is provided on pressure and pinching of the skin. Activation of a descending inhibitory pathway (HOLMQVIST *et al.*, 1960 a) suppresses the effects from muscle afferents converting this tract to an exteroceptive pathway

3) A pathway excited by cutaneous and by high threshold muscle afferents after a long central delay Strong excitatory and inhibitory effects are evoked on stimulation of the anterior cerebellum.

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Acta physiol. scand. 1961 51 17—28

From the Neurophysiological Laboratory at the Anatomical Institute, University of Oslo, Norway

Activation of the Dentate Area by Septal Stimulation

By

PER ANDERSEN, HELGE BRULAND and BIRGER R. KAADA

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Abstract

ANDERSEN P H BRULAND, and B. R. KAADA, *Activation of the dentate area by septal stimulation.* Acta physiol. scand. 1961 *51* 17—28. — In rabbits under urethane-chloralose anesthesia potentials were recorded bilaterally from the dentate area in response to stimulation of the large celled posterior part of the medial septal nucleus. The septo-dentate response consisted of an initial negative spike followed by slower positive wave. On the basis of recordings from the various cell layers, excitability properties, resistance to anoxia and repetitive stimulation, the initial spike is interpreted as discharges of the dentate granule cells, monosynaptically excited. The following positive wave may partly represent the subsequent spread of excitation along the dendrites of the granule cells, and partly the activation of CA4 neurons. — Stimulation of the contralateral field CA3 of the hippocampus evoked within the dentate area a potential similar in form, but with a greater amplitude and shorter latency than the septo-dentate response. This crossed CA3-dentate potential is similarly most likely due to activation of the same granule cells. The crossed projection appears to be more efficient and consists of thicker fibres than the septo-dentate path.

In the attempts to elucidate the functional role of the hippocampal region increased knowledge of the sources of the afferent impulses to this region is needed. In a study of the projections from the septum it was found that single

The term hippocampal region is used in the same sense as by BLACKSTAD (1956): It includes the hippocampus, area dentata, subiculum, presubiculum, parasubiculum, area entorhinalis, and area retrosplenialis. The area dentata includes the fascia dentata (the molecular and granular layers) and hilus fasciae dentatae. The latter corresponds to CA4 of LORENTE DE NÓ (1934)

shock stimulation of this area in rabbits produced within the hippocampal region two distinct types of potentials, one recorded from the dentate area, and a second from the ventricular surface of field CAI, whereas no distinct potentials were obtained from the subicular and presubicular areas.

The anatomical reports on projections from the septal areas to the fornix and hippocampal region contain several controversial points which need further experimental clarification. These concern the existence of true septo-hippocampal and septo-dentate fibres as well as their exact origin course and termination (for references see DAITZ and POWELL 1954 McLARDY 1955 a, b, CRAGG and HAMLYN 1957 VOTAW 1960)

The present investigation represents an attempt to clarify some of these controversial issues and to study the mechanism of action of the presumable septo-fugal impulses on the dentate neurons. In a subsequent article observations concerning the septo-hippocampal projection will be presented (ANDERSEN, KAADA and BRULAND 1960)

In an independent investigation, EULER and GREEN (1960 a, b) have studied the same projections. The present study corroborates some of their findings and gives additional information concerning the origin and distribution of the septo-dentate connections.

Material and Methods

Sixteen adult rabbits anesthetized with urethane-chloralose[1] (750 and 40 mg/kg respectively) intraperitoneally were used. The hippocampus and septum were exposed by suction of the overlying grey and white matter and covered by warm mineral oil.

Stimulation was performed with bipolar stainless steel electrodes using square wave pulses of 0.1 msec duration, a frequency of 0.3/sec, and intensities varying between threshold and five times threshold values. The recording electrodes consisted of a stainless steel wire (20—30 μ) or micropipettes (about 5μ) filled with a 3 M KCl solution. The records were obtained monopolarly using a conventional push-pull amplifier

Anoxia was produced in animals immobilized by intravenous decamethonium bromide (Decacurin® AFI) in doses of 0.25 mg/kg by replacement of the oxygen by nitrogen through the respirator

The electrode tracks were identified using a modified *Nauta* method (Andersen 1956) The histological picture was compared with the potentials obtained from the various depths. The same staining technique was used in experiments with fibre sections to determine the extent of the lesions.

Results

Pattern of response — Following stimulation of the septal region a typical potential was recorded bilaterally from the dentate area. This consisted of an initial sharp negative spike, followed by a slower positive wave of greater ampli

[1] The chloralose was kindly supplied by E. Merck, Darmstadt.

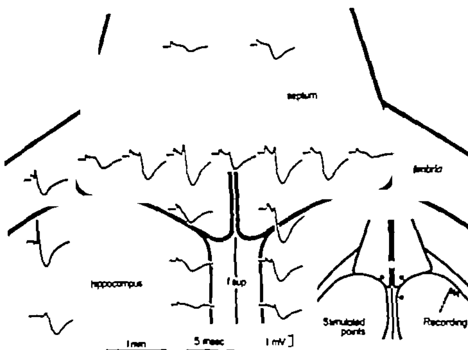


Fig 1 *Origin of the septo-dentate projection.* Stimulation of the dorsal surface of the septum, fimbria, and hippocampus and recording from the dentate area. The potentials obtained transferred to the corresponding stimulated points as indicated by the key at the lower right.
I all figures the potentials are recorded monopolarly with negativity upwards.

tude (Fig 1) Sometimes a late negative spike was superimposed on the positive wave. However this spike was shown to represent the discharge of CA1 cells and will therefore not be dealt with further The latency of the initial spike measured 2—5 msec and the duration 2—4 msec. The duration of the positive wave ranged from 10 to 25 msec and its amplitude amounted up to 7 mV This slow wave was sometimes followed by a negative wave of lower amplitude and of a duration of 50—100 msec.

The threshold of the initial spike and the positive wave was about the same, whereas the negative wave appeared on higher stimulus strengths. On increasing intensities the positive wave showed a considerably greater increase of the amplitude than did the spike.

When a small glass capillary electrode was used for recording, the initial negative spike was at certain depths replaced by multiple small spikes (Fig 3 D)

Occasionally the potential obtained at certain depths of the dentate area consisted of a pure negative wave of 7—15 msec duration and with a slightly longer latency than the initial negative spike (Fig 4)

Delimitation of low-threshold structures in the septal region. — Because of the histolog

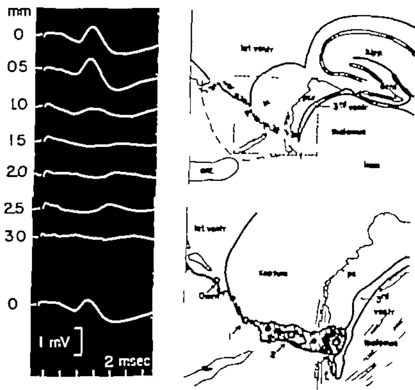


Fig 2. *Origin of the septo-dentate projection.* Left, stimulation at indicated depths (in mm) within the septal region and recording from a fixed point in the ipsilateral dentate area. Right, drawings from sagittal section 0.5 mm lateral to the midline demonstrating the track of the stimulating electrode in relation to adjacent structures.

ical complexity of the region stimulated it was first necessary to determine which elements were responsible for the potentials evoked in the dentate area.

By weak stimulation of the dorsal *surface* of the septum and the adjacent portions of the hippocampus and fimbria the typical response was recorded bilaterally in the dentate area when the electrode was placed on the postero-medial part of the septum (Fig 1) Stimulation of the antero-lateral portion of the septum produced only smaller potentials. Stimulation in the midline was less effective than was stimulation 0.5 mm laterally Inconsistent and small dentate responses were evoked from the ipsilateral fimbria and field CA3 of the hippocampus. On the other hand, excitation of the contralateral field CA3 produced a dentate potential which was similar in form but of considerably shorter latency and higher amplitude than that elicited by septal stimulation (cf. below)

Fig 2 shows the results obtained by stimulation at various *depths* within the septal region and recording from the dentate area Maximum responses were obtained when the tip of the penetrating stimulus electrode was situated within

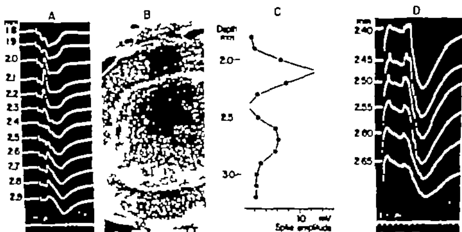


Fig. 3. *Distribution of the septo-dentate response.* Medial septal stimulation, recording at indicated depths within the ipsilateral dentate area. *A* shows two maxima of the initial spike, corresponding to the two blades of the granular cell layer in *B*. *C*, variation in amplitude of the spike at the various depths. *D* shows that the initial spike may be composed of a number of smaller spikes, and that these are recorded from a narrow zone only.

the dorsal 1 mm of the septal nuclei, whereas the response disappeared when the tip was inserted into the fornix (f) or the ventral psalterium (ps. v).

In conclusion, these results suggest that the septal structure yielding rise to the dentate potentials is the postero-dorsal part of the medial septal region, corresponding to the location of the magnocellular medial septal nuclei (YOUNG 1936) on both sides, and not the fornix or ventral psalterium.

Course of the septo-dentate projection. — The septo-dentate response was left unaltered by section of the fornix superior, the fimbria and the alveus between the stimulating and recording electrodes. Therefore, the fibres mediating the response is apparently situated deep to the ventricular surface of the hippocampal region, and is located between the most medial and the most lateral parts of the fornix system. Destruction of the septal nucleus on one side did not significantly affect the septo-dentate potentials recorded on either side, indicating that the projection is bilateral.

Distribution of the septo-dentate impulses. — Consistent modifications of the septo-dentate response were encountered as the recording electrode penetrated the various layers of the dentate area from the dorsal side (Fig. 3). First, the amplitude of the initial spike showed two maxima at two different recording depths, the distance between these measured from 0.6 to 0.8 mm along the usual electrode track. These maxima appear to correspond to the upper and lower blades of the granule cell layer (Fig. 3 B). When the initial single spike was replaced by a series of smaller spikes (Fig. 3 D), the amplitude of each individual

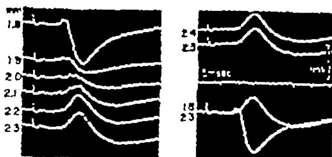


Fig 4. Relation between the initial spike and the following wave of the septo-dentate response Records obtained at indicated depths within the dentate area. In the last record the potentials obtained at 1.8 and 2.3 mm are superimposed.

spike showed the same change at the various depths, their number being constant. Second, the polarity of the initial spike changed as the electrode penetrated the dentate area. At a depth presumably corresponding to the upper blade of the granule cell layer the spike was almost purely negative (Fig 3 A B 2.1 mm below the ventricular surface) Within the hilus of the dentate area the spike was almost absent (2.4 mm) At a still deeper level (2.7 mm) probably corresponding to the lower blade of the granular layer the spike was again almost purely negative From these observations it may be concluded that the initial negative spike of the septo-dentate response probably represents the discharge of the granule cells of the dentate area.

As mentioned above the usual dentate response to medial septal stimulation, i.e. the negative spike followed by a positive wave was in some experiments at certain depths replaced by a single negative wave. The relation between the two types of septo-dentate response at various depths appears from Fig 4 At a depth of 1.8 mm below the ventricular surface, corresponding to the upper blade of the granular layer the response was of the ordinary type. At deeper levels the polarity of the positive wave was reversed. The pure negative wave recorded from the central and deeper parts of the dentate area had a longer latency than the negative spike recorded from the upper blade of the granular layer as seen in the last record.

In conclusion, it may be stated that the septo-dentate response ordinarily consists of two components, an initial negative spike (representing the summation of several individual spike discharges) followed by a positive wave of longer duration. Sometimes the deeper parts of the dentate area may yield a response that consists only of a slow negative component. The initial spike and the subsequent wave — whether recorded positive or negative and isolated — are most likely due to the activity in two different structural elements within the dentate area. This assumption is further corroborated by the experiments to be described below



Fig. 3.

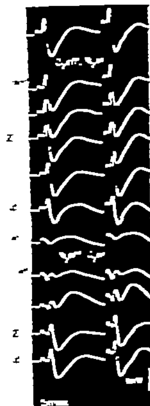


Fig. 4.

Fig. 3. *Excitability changes of the septo-dentate projection as tested by repetitive stimulation. A*, post-tetanic potentiation affecting the positive wave only. *B*, post-tetanic depression, after a series of tetani, influencing the positive wave to a greater extent than the initial spike.

Fig. 4. *Effects of* [illegible] *on the septo-dentate response.* Paired shocks delivered to the medial septal area. The positive wave is reduced before the initial spike and is the last to appear on [illegible] of [illegible].

Excitability properties of the septo-dentate response. — The two components of the typical septo-dentate response behaved similarly when tested by paired shocks. The initial negative spike showed a moderate facilitation at delays from about 30 to 100 msec and was diminished at shorter delays. The absolute refractory period of this component measured 1.5—2 msec. The positive wave showed a similar facilitation and depression at the corresponding delays.

By repetitive stimulation the two main components of the septo-dentate response behaved differently. The amplitude of the initial spike was only little affected by stimulus frequencies up to 10/sec. In contrast, the following positive wave was often markedly depressed (Fig. 3 B) even at repetition rates as low as

5/sec. This depression was sometimes associated with an increase in duration of the initial spike.

Following a burst of repetitive stimuli of relatively short duration (5—15 sec) and moderate frequency (10—20/sec) the positive wave exhibited a distinct post tetanic potentiation, whereas the initial spike was almost unchanged (Fig 5 A) As typical for post tetanic potentiation, its degree and duration was enhanced with increasing rate and duration of the tetanic stimulation provided these values were not too high so as to produce depression. The post tetanic potentiation lasted from 5 sec to 3 min

More intense repetitive stimulation or a series of tetanic stimulation periods, resulted in a depression of all components of the septo-dentate response, lasting from a few seconds to 5—10 min The initial spike was the most resistant part of the response (Fig 5 B) *The restitution of the septo-dentate response subsequent* to a depression followed a typical sequence First, the initial negative spike and then the positive wave gradually reappeared.

Spontaneous self-sustained discharges were seldom recorded from the dentate area as compared to the hippocampus. Those obtained from the dentate electrode consisted of positive and fairly slow waves. These were interpreted as discharges originating at some distance most probably in the hippocampus. Simultaneous recording from the dentate area and from the field CA1 of the hippocampus corroborated this assumption. Also, afterdischarges following septal stimulation were more readily recorded from the pyramidal layer of the hippocampal field CA1 where they appeared as sharp negative waves, whereas the dentate electrode only recorded the distant, positive and slower potentials.

Effects of anoxia on the septo-dentate response By anoxia a further distinction was made between the two main components of the septo-dentate response. The initial negative spike was more resistant to anoxia than was the positive wave (Fig 6) The latter was sometimes replaced by a slow negative wave In one experiment the spike was recorded for as long as 23 minutes during pure nitrogen breathing under over pressure its amplitude remained constant for about 4 minutes, followed by a gradual decrease. By readmission of oxygen full restitution took place within 2 minutes. The duration of the initial spike increased somewhat during the anoxic period. This was apparently due to the abolition of the subsequent positive wave. This demasking of the initial spike resembles the results obtained by repetitive stimulation (Fig 5 B)

Relation between the activation of the dentate area by ipsilateral septal and by contralateral CA3 stimulation. The dentate response to stimulation of the septum and the contralateral field CA3 (the crossed CA3-dentate response) are shown in Fig. 1 and 7 The two potentials are similar in form. However in spite of the longer conduction distance (as measured along the course of the ventral psalterium fibres) the latency of the crossed CA3-dentate response was considerably shorter than that of the septo-dentate response (Fig 7) Furthermore the amplitude of the crossed CA3-dentate potential was higher By placing the

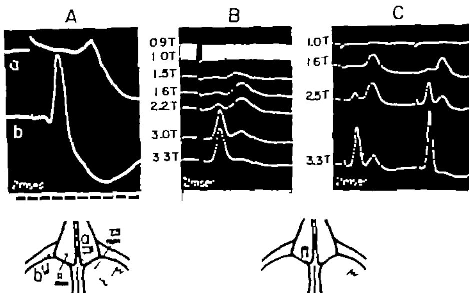


Fig. 7 *Relation between the septo-dentate and the crossed CA3-dentate response.* A, stimulation of ipsilateral medial septal region (a) stimulation of the contralateral CA3 (b). Recording from the same point within the dentate area. The conduction distances 7.5 and 11 mm respectively. — B stimulation at increasing stimulus strengths (T = threshold) near the border between the septum and the hippocampus recording from the contralateral dentate area. — C, electrode arrangement as in B. Paired shocks at given interval with increasing intensities. Facilitation of the first, high-threshold spike of the CA3-dentate response and depression of the second, low-threshold spike of the septo-dentate response.

stimulating electrode on the caudal part of the septum, near the border to the hippocampal field CA3 the latter field could be excited by strong stimulation (Fig 7 B and C) Weak stimulation (1—1.5T) elicited the negative spike of the septo-dentate response, which had a duration of 3.5 msec and a latency of 4.2 msec. By stronger stimulation (1 6 T) a new spike of 2.5 msec duration and a latency of only 1.8 msec appeared, probably due to spread of the stimulating current to the adjacent field CA3 Still stronger stimulation produced an augmentation of the short latency spike, whereas the late one was depressed and ultimately completely abolished. Since occlusion occurred when the stimulus strength was increased (Fig. 7 C) the two spikes probably represent the activity of the same elements evoked by afferent impulses through the two routes.

During anoxia the septo-dentate as well as the crossed CA3-dentate response showed within 1 or 2 min a depression of the late spike and the positive wave, whereas the initial spikes of both responses resisted anoxia for more than 10 min, although their amplitudes gradually diminished towards the end of this period. The high and similar resistance to anoxia of the initial spike of the two responses in question is also in favour of the assumption that the activation is a direct one for both afferent routes.

Discussion

Localization of the low-threshold structures within the septal region. The results presented indicate that the potential recorded from the dentate area on stimulating the septal region probably is due to the activation of neurons lying in the posterior dorso-medial part of the septal nuclear mass on both sides, possibly the magnocellular medial septal nuclei. The fibres of the fornix and the ventral psalterium seem to have a higher threshold and are probably not stimulated directly by the weaker shocks. This interpretation of the origin of septo-fugal impulses coursing posteriorly into the fornix is in agreement with anatomical observations by METTLER (1945), ROSE and WOOLSEY (1943), DAITZ and POWELL (1954) and MCLARDY (1955 a). In these studies retrograde cell changes were found in the medial septal nucleus following damage to the hippocampus, fimbria or fornix.

Course and distribution of the septo-dentate impulses. The section experiments suggest that the septo-dentate impulses course along the fibres between the most medial bundles within the fornix system (the fornix superior) and the lateral ones forming the fimbria. This is in good agreement with the results presented by MCLARDY (1955 b) who stated that the medial fifth of the body of the fornix contained fibres that penetrated the corpus callosum to join the cingulum whereas the middle three fifths enclosed efferent fibres from the magnocellular part of the medial septal nucleus ending either in the hippocampus or in the temporal neocortex.

The recording from the various layers of the dentate area suggests that the septo-dentate projection activates two distinct zones apparently corresponding to the two blades of the granule cell layer. There were additional signs of activation of cells lying in the hilus of the dentate fascia (see below). Stimulation of a single point within the posterior medial septal region caused a fairly widespread activation of the dentate granule cell layer. The experiments do not allow any conclusions regarding a possible topographical relationship between different parts of the medial septal nucleus and the various portions of the dentate area.

Mode of activation of the granule cells by septo-dentate impulses. The initial negative spike of the septo-dentate response probably represents discharges of the granule cells. It seems permissible to conclude that the single spike most likely is composed of the nearly synchronous discharges of a group of granule cells, whereas the multiple smaller spikes recorded with the micropipettes represent the firing of individual or of a small number of such cells. The short refractory period (1.7—1.8 msec) and the great resistance to anoxia suggest that the granule cells are excited monosynaptically by the septo-dentate path as previously indicated by EULER, GREEN and RICCI (1958).

The interpretation of the following slow wave is more difficult. This was recorded as a positive wave from the granule cell layer and usually also from the hilus fasciae dentatae (CA4). However sometimes it was negative in the latter region. Thus the wave may reflect the activity of two different elements.

One component of this wave may represent retrograde invasion of the depolarization from the soma into the dendritic tree of the granule cells, the other may be due to excitation of CA4 neurons. The depression of the positive wave by anoxia and by repetitive stimulation may be due to a block of this dendritic activation. The usual immense positivity of the slow wave when recorded from the hilus fasciae dentatae is also in favour of the interpretation of this component as partly due to spread of the excitation from the soma of the granule cells along their dendrites. The positivity and the high amplitude might be explained on the basis of the histological arrangement in the dentate area with the granular and molecular layers enclosing the hilus, through which the efferent axons of the granule cells, the mossy fibres, are coursing an orientation resembling the closed field of LORENTE DE NÓ (1933)

In conclusion, the slow wave following the initial spike may be due partly to the activation of granule cell dendrites and partly to the excitation of CA4 neurons.

Relation between the ipsilateral septal and the crossed CA3 activation of the granule cells. The most striking feature when comparing these two routes of dentate activation is the similarity in form of the evoked potentials. The mutual occlusion of the negative spike indicates that the same elements are activated by the two afferents. Strong evidence is offered by experimental histological work that interhippocampal impulses activate the granule cells (BLACKSTAD 1956) This strengthens the assumption that the initial spike of the two responses represents the activity of granule cells. However when comparing the two responses, it is evident that stimulation of the contralateral CA3 is the more effective one, producing a much larger response with a considerably shorter latency than that elicited by ipsilateral septal stimulation. The great amplitude of the spike evoked by contralateral CA3 stimulation indicates a high degree of efficiency of this afferent route. This is in agreement with the massive terminal degeneration around the proximal part of the granule cell dendrites following severance of the contralateral hippocampal formation (BLACKSTAD 1956) The shorter latency of the crossed CA3-dentate response suggests that the fibres mediating this response are thicker than those conveying the septo-dentate impulses.

The absence or scarcity of sustained electrical afterdischarges in the dentate area may possibly be explained on the basis of the different structural organization between this area and the hippocampus where such discharges are very common. The main difference seems to be the smaller possibilities for repetitive stimulation by way of recurrent collaterals within the dentate area.

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Acta physiol. scand. 1961 51 29—40

From the Neurophysiological Laboratory t the Anatomical Institute,
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Activation of the Field CAI of the Hippocampus by Septal Stimulation

By

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Received 8 August 1960

Abstract

Andersen, P H. Bruland and B. R. Kaada. *Activation of the field CAI of the hippocampus by septal stimulation.* Acta physiol. scand. 1961 51 29—40. — In rabbits under urethane-chloralose anaesthesia the existence of septo-hippocampal projection has been verified by the evoked potential method. Its origin is the magnocellular medial septal nucleus. These septo-fugal fibres activate the basal parts of the apical dendrites and/or the soma of the CAI neurons, probably by mono-synaptic route By repetitive stimulation and by strychnine the depolarized area of the CAI neurons may increase, by anoxia it may decrease. Thus, according to the excitability level, smaller or greater part of the apical dendrites may initiate spikes of an all-or-none character

In a previous communication (Andersen, Bruland and Kaada 1960) the activation of the dentate area by stimulation of the medial septal nucleus was reported. The present article is concerned with the activation of the field CAI of the hippocampus in response to stimulation of the same structure.

Material and Methods

Seventeen adult rabbits anesthetized with urethane-chloralose[1] were used. Detailed description of the technique has been given in previous communication (Andersen Bruland and Kaada 1960) I brief, the evoked potential technique was used employing small stimulation and recording electrodes. The analysis of the responses were made by depth recording and stimulation, and by the use of paired and repetitive stimuli, anoxia and locally applied strychnine.

[1] The chloralose was kindly supplied by E. Merck Darmstadt

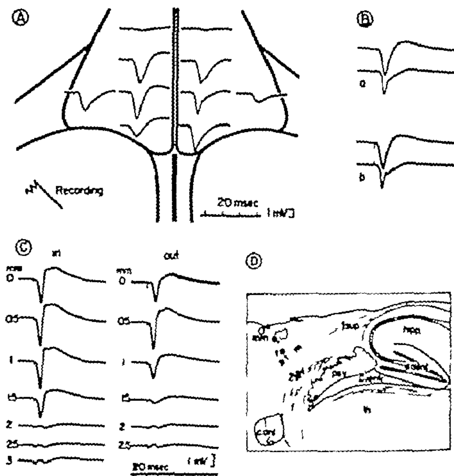


Fig. 1. *Origin of the septo-hippocampal projection. A.* Stimulation of various points on the dorsal surface of the septal region. *The potentials recorded from the left hippocampus are transferred to the respective stimulation points. B.* Records obtained (a) before and (b) after suction of the septum of one side (left). Upper beam ipsilateral (right), lower beam contralateral (left) records. C. Potentials obtained from the surface of CA1 in response to stimulation at different depths as indicated in *D. In* shows records observed on insertion, *out* on retraction.

In all figures monopolar recordings and negativity upwards.

Abbreviations

a. dent.	dentate area	hipp.	hippocampus
c. ant.	anterior commissure	ps.	psalterium
f.	fornix	th.	thalamus
f. sup.	superior fornix	III ventr.	third ventricle

Results

Stimulation of the exposed dorsal surface of the septum elicited a typical potential in the ipsi- and contralateral fields CA1 of the two hippocampi. The response consisted of a positive negative wave with one or two negative spikes superimposed on the positive wave (Fig. 1 and 2). The response thus resembles

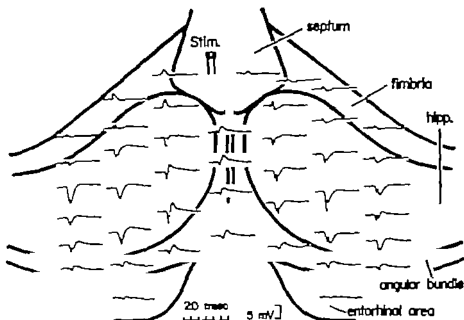


Fig. 2. Distribution of the septo-hippocampal potentials. The distribution is bilateral. Note the small potentials in CA3 (near the fimbria) over the angular bundle and in the entorhinal area.

the CA1 commissural potential evoked by stimulation of the symmetrical point of contralateral field CA1 (ANDERSEN 1959) *The slow surface positive and* negative waves had the same threshold, whereas that of the spikes was higher

In order to delimit the structure w thin the septal region which gave rise to the hippocampal potentials, stimulation was performed at different surface points and at various depths through an electrode penetrating the septal nuclei, the fornix, and the ventral psalterium. The results are presented in Fig 1 The excitable area was found to be situated in the postero-medial, dorsalmost part of the septum, corresponding to the magnocellular part of the medial septal nucleus (YOUNG 1936) No typical potential could be recorded when the stimulating electrode was placed within the fornix or the ventral psalterium. Thus, the projection in question appears to originat in the medial septal nucleus and represents a true septo-hippocampal pathway

To determine whether the contralateral potential was a direct one or mediated via the septal nuclei of the opposite side, records were obtained bilaterally before and after suction of the septal nuclei of one side (Fig 1 B) The upper record, in each pair is the psilateral response whereas the lower shows the contralateral response. The persistence of the potentials indicates that both projections are direct.

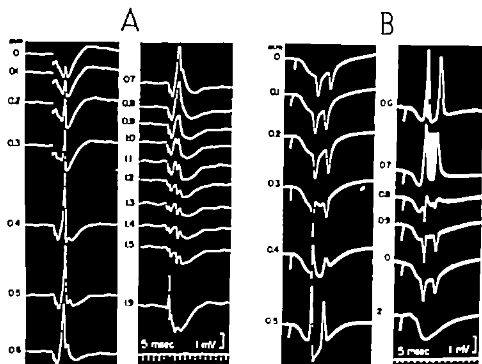


Fig 3 *Depth recording* Potentials obtained with glass capillary electrodes at indicated depths of CA1 on medial septal stimulation. A and B from two different experiments. Last records in A and B from dentate area for comparison.

Course of the septo-hippocampal pathway Section of the fimbria and the exposed midline structures, including the fornix longus and the medial part of the fornix proper were without effect on the typical CA1 potential. On the other hand, a lesion confined to the alveus, severing the white matter between the septo-hippocampal border and the recording electrode abolished the response It is concluded that the septo-hippocampal fibres are situated between the most medial and lateral parts of the fornix fibre system and then course in the alveus almost directly to the dorsalmost part of CA1. Fibres to more lateral regions of this field probably follow the fimbria for a short distance before merging into the alveus.

Distribution of the septo-hippocampal projection. Surface recording Stimulation of the postero-medial part of the septum elicited potentials distributed over a wide area of the dorsal hippocampus (Fig 2) The area of maximal responses was found to be the CA1 Potentials of smaller amplitude were recorded from the CA3 their form was different from the typical CA1 response being mainly surface negative *Depth recording* By this procedure the spikes were found to have their greatest amplitude in the pyramidal layer (0 4—0.5 mm) and in the adja-

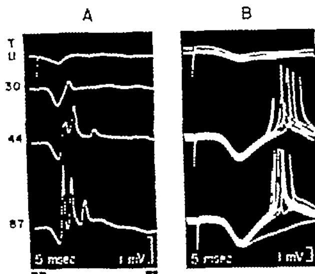


Fig. 4. *Relation between the deep negative wave and the spike. A.* Records obtained at 0.6 mm depth with increasing stimulus strength. T = threshold. *B.* Records obtained from 0.6 mm depth during restitution from a tetanus-produced depression. Further explanation in text.

cent part of the stratum radiatum (Fig. 3). In the latter region a substantial part of the surface-positive wave was recorded with reversed polarity. In some experiments, however, the positive wave was diminished but not reversed at 0.4—0.7 mm (Fig. 3 B). For reasons given below the deep negative wave is regarded as an excitatory postsynaptic potential elicited at the basal parts of the apical dendrites, suggesting that this part of the CA1 neurons is the main site of termination of the septo-hippocampal projection. Following single shock stimulation, negative spikes were not recorded from the rest of the stratum radiatum, *suggesting that the spikes during such conditions are not conducted somato-fugally along the apical dendrites.* This finding is in agreement with the results obtained by EULER, GREEN and RICCI (1958).

The relation between the spikes and the slow negative wave recorded from about 0.6 mm below the surface is elucidated by Fig. 4. *A* shows the effect of increasing stimulus strength. On increasing intensity the negative wave was augmented and one or more negative spikes occurred superimposed on it. The greater the negative wave the more spikes appeared. *B* shows three records taken during the restitution following a period of depression as a result of tetanic stimulation. The development of the negative wave (recorded from a depth of 0.6 mm) is associated with the appearance of spikes. These emerge from a given level of the negative wave, their latencies being shorter at the end of the restitution period as compared with the start.

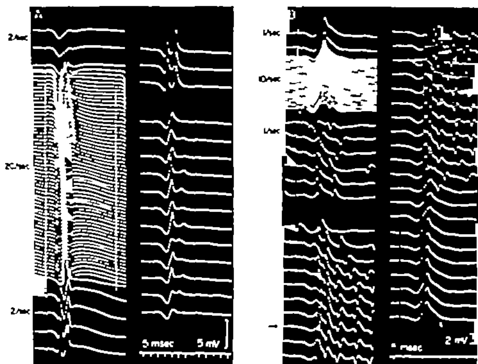


Fig 5 *Effects of tetanic stimulation.* A Surface CA1 records obtained t indicated stimulating frequencies. I right column the open space between the third and fourth sweep represents an interval of 18 sec (36 sweeps) Note change in latency of the spikes during and af er th tetanic stimulation. — *B* Records obtained 0.6 mm below the surface of field CA1 t indicated frequencies. Each record is shown, except that 5 sweeps (5 sec) have been omitted i the open space 7 sec af er the tetanus Repetiti discharges from about 12 sec af er the tetanus. Arrow ndicates the record which shows the purest negativity of the first spike of the repetitive discharges

Excitability changes f the CA1 neurons produced by septal stimulation. Paired shocks delivered to the medial septal nucleus showed an enhancement of the test response, especially of the spike at delays from about 10 to 200—300 msec. At delays shorter than 10 msec the test potential was diminished total abol tion occurred at a delay of about 4 msec, probably representing the absolute refractory period of the CA1 neurons activated by the septo-hippocampal impulses.

Tetanic septal stimulation caused several alterations of the CA1 response These consisted of changes in the latency and in the ampl tude of the spike, in the production of new spikes, or of afterdischarges.

In the experiment illustrated in Fig 5A repetitive stimulation enhanced the amplitude of the first spike and elicited a new spike after the first one This second spike was much more susceptible to tetanic stimulation being quickly abolished after the initial enhancement. During such stimulation the latency of the initial spike t first decreased then it gradually increased and reached

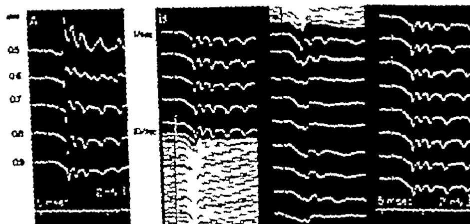


Fig. 6. *A*. Repetitive discharges recorded from indicated depths below the surface of CA1. *B* Effect of tetanic stimulation on the repetitive discharges recorded 0.8 mm below the surface. Time interval between first and second columns of *B* is 10 sec. See text.

the prestimulatory value. The first single shock after the tetanus elicited a spike with a minimal latency and maximal amplitude, but after a while it gradually changed to the original form and time course. Principally similar results were observed with regard to the second spike.

Post tetanic potentiation occurred almost regularly affecting the spikes as well as the late surface-negative wave (Fig. 5A). The duration ranged from some seconds to about 5 min, depending upon the frequency and the duration of the tetanic stimulation. Occasionally a spike could be produced by post tetanic potentiation. Excitation at a frequency above 50/sec often resulted in a pure depression of the potentials which outlasted the stimulation by several minutes. The first component to reappear was the surface-positive wave, followed by the surface-negative one then the first and the second spikes returned.

In the period following a tetanus of relatively short duration or low frequency each single shock could elicit repetitive discharges (Fig. 5B and Fig. 6). These probably represent a sign of increased responsiveness of the CA1 cortex because this condition often preceded one in which tetanic stimulation produced the usual long-lasting and slow afterdischarges. The first spike of such repetitive discharges underwent a gradual change in configuration — when recorded from 0.6 mm below the surface (Fig. 5B). In the period immediately following the tetanic stimulation the repetitive discharges were irregular but after about 15 sec they became more uniform. The discharges consisted of diphasic spikes first increasing, then decreasing in number. Concomitantly with the increased number of discharges the first spike changed from a positive/negative polarity to a pure negativity (at arrow) and subsequently resumed its original form. The

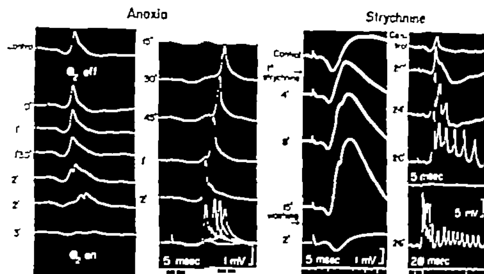


Fig. 7. *Effects of anoxia and strychnine on the septo-hippocampal potential* (see text). Effect of 3 min anoxia on the potential recorded 0.7 mm below the surface of CA1. Note the change in spike polarity at 2 min. The second column shows the restitution with the augmented spike superimposed on a slow negative wave. In the last record the sweeps obtained during the restitution period are superimposed to demonstrate the relation between the slow negative wave and the spike. — *Strychnine*. Left column, surface records. Augmentation of surface negative wave with development of several spikes. Right column, records obtained 0.7 mm below the CA1 surface. Strychnine applied at arrow. Increase of the deep negative wave and of amplitude and number of spikes. Last record obtained with slower time base to show the rhythmical and long-lasting discharge.

remaining spikes within the group were all positive/negative but the positivity was smallest when the first spike was purely negative.

Repetitive discharges recorded from different levels of CA1 are illustrated in Fig. 6A. At the pyramidal layer (0.5 mm) the spikes are purely negative and superimposed on a slow negative wave. At deeper levels the spikes become diphasic, suggesting propagation of impulses from the cell bodies along the apical dendrites. At 0.9 mm the spikes are purely positive, suggesting a conduction block at this site.

Fig. 6B shows the effect of tetanic stimulation (10 sec) on the repetitive discharges recorded from the apical shaft layer (0.9 mm deep). The stimulation was followed by a period of depression lasting for about 20 seconds. At this low stimulating frequency no invasion of repetitive discharges into the apical dendrites was noted, as found by EULER et al. (1958). Higher frequencies produced such invasion, but from the layer of the terminal branches of the apical dendrites only positive potentials were recorded.

Effect of anoxia and strychnine. Deprivation of the oxygen supply resulted in the disappearance of the spike in 2—2½ min *per pausa* with a gradual and slow decrease of the negative wave recorded from a depth of 0.7 mm (Fig. 7). *Before*

the abolition of the spike its latency increased and the polarity shifted from pure negativity to positive/negative diphasicity a phenomenon similar to that observed for the initial spike of the repetitive discharges (Fig 5B) On readmission of the oxygen the spike occurred with increased amplitude and prolonged latency superimposed on a slow negative wave. The further development was characterized by an augmentation of the negative wave and a decrease of the spike latency This experiment shows that the spike initiating mechanism is ready for operation shortly after an anoxic period, and that the restitution of the mechanism for the triggering of the spike — probably the deep negative wave — is the essential factor in the establishment of a normal response.

Locally applied strychnine sulfate (a piece of filter paper soaked in a 1 per cent solution) produced the reverse of the anoxia effect (Fig 7) The surface negative waves and the deep negative wave were markedly increased in magnitude as well as in duration and a series of negative spikes occurred superimposed upon them. Strychnine increased also the first spike (last column) When this was absent, strychnine could elicit a spike of the same latency as that of the usual first spike.

Discussion

The existence of a septo-hippocampal projection. As mentioned, the recorded hippocampal potentials are most likely due to excitation of the magnocellular medial septal nucleus. In addition, it cannot be excluded that the nucleus septo-hippocampalis (Young 1936) contribute to the projection as well. The failure to produce potentials, with the same stimulus strengths, when the stimulating electrode was situated within the fornix or the ventral psalterium, indicates that these fibre bundles have a higher threshold, and that excitation of those fibres plays a minor role, if any in eliciting the recorded hippocampal potentials. Thus, the experiments suggest the existence of a true septo-hippocampal pathway which originates bilaterally in the medial septal nuclei. Similar conclusions have been arrived at with regard to the septo-dentate projection (Andersen *et al* 1960) This interpretation is in essential agreement with studies on retrograde cell changes within the medial septal nucleus following destruction of the hippocampal formation (Mettler 1943 Rose and Woolsey 1943 Daitz and Powell 1954 McLardy 1955) and normal anatomical data (Young 1936) According to Mettler, Daitz and Powell the projection is ipsilateral, whereas McLardy found degeneration on both sides following unilateral fornical section.

Several authors have described potentials recorded from the hippocampus in response to dorsal fornix stimulation (Green and Adey 1956 Dunlop 1957 Euler *et al* 1958) In view of the high threshold of the fornix fibres compared with that of the medial septal nuclei it appears likely that they in fact have excited the latter nuclei. This would explain the surprisingly low chronaxie which Green and Adey (1956) found by such stimulation. These authors state

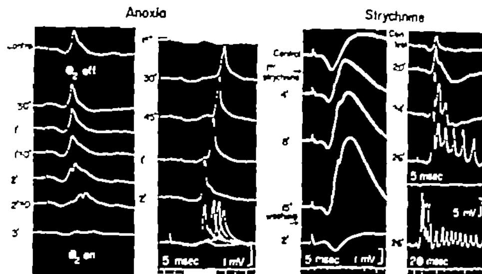


Fig. 7. *Effect of anoxia and strychnine on the septo-hippocampal potential. Anoxia.* Effect of 3 min anoxia on the potential recorded 0.7 mm below the surface of CA1. Note the change in spike polarity at 2 min. The second column shows the restitution with the augmented spike superimposed on a slow negative wave. In the last record the sweeps obtained during the restitution period are superimposed to demonstrate the relation between the slow negative wave and the spike. — *Strychnine.* Left column, surface records. Augmentation of surface negative wave with development of several spikes. Right column records obtained 0.7 mm below the CA1 surface. Strychnine applied at arrow. Increase of the deep negative wave and of amplitude and number of spikes. Last record obtained with slower time base to show the rhythmical and long-lasting discharge.

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Effect of anoxia and strychnine. Deprivation of the oxygen supply resulted in the disappearance of the spike in 2—2 1/2 min *pari passu* with a gradual and slow decrease of the negative wave recorded from a depth of 0.7 mm (Fig.) Before

with the assumption that the septo-hippocampal fibres terminate on the border between the soma and the apical dendritic shafts.

Excitability properties of the field CA1 as studied by the septo-hippocampal impulses. The ease with which electrical afterdischarges and post tetanic potentiation may be produced within CA1 stands in a marked contrast to the conditions found in the dentate area (ANDERSEN *et al.* 1960). This difference may probably be explained by a different synaptical arrangement.

Of special interest is the peculiar variations in latency observed from CA1 during and after a period of tetanic septal stimulation (Fig. 5A). The increase of the spike latency after the initial shortening during the tetanus does not seem to be directly related to the mechanism responsible for the production of the spike since it was recorded with unaltered amplitude both during and after the tetanic stimulation. Therefore, the increase in the spike latency is probably due to a depression of the process leading to the spike discharge, the synaptic transmission, rather than the mechanism of spike discharge itself.

Some of the observations concerning the repetitive discharges deserve further comments. The temporary change in configuration of the first spike of the series of discharges from diphasicity to pure negativity (Fig. 5B) suggests that the spike took its origin at a certain distance from the electrode in the beginning of the period, whereas it started close to the electrode tip at the height of the period of increased excitability. The most probable site of the initiation of the spike by septo-hippocampal impulses under normal conditions is the cell body. In states of increased irritability, however, this area may increase and the spike can then take its origin from a greater area of the neuron, including part of the apical dendrites.

Conversely, anoxia was able to change a potential obtained from just below the pyramidal layer from negativity to positivity/negativity. This phenomenon could similarly be explained by a narrowing of the depolarized area during anoxia, from one comprising the soma and a part of the dendrites to one restricted to the soma alone.

The degree of excitability would then be directly related to the area of the neuronal cell membrane that the afferent impulses depolarize. The more the excitability is enhanced above the normal level, the greater is the part of the dendrites which may be invaded by the spikes initiated in the soma, as observed by EULER et al. (1958) and by EULER and GREEN (1960b). The repetitive spike discharges described above (Fig. 5B) may represent a transition between the normal condition and one of highly increased excitability resulting in prolonged electrical afterdischarges or in spontaneous, self-sustained epileptiform discharges.

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Does the Urinary Excretion of Imidazole Acetic Acid Reflect the Endogenous Histamine Metabolism in Man?

By

Hans Duner, Sten Otto Liljedahl and Bengt Pernow

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Abstract

Duner, H., S. O. Liljedahl and B. Pernow. *Does the urinary excretion of imidazole acetic acid reflect the endogenous histamine metabolism in man?* Acta physiol. scand. 1961. *51* 41—46. — The urinary excretion of free and conjugated histamine, histidine and imidazole acetic acid (ImAA) was studied in four healthy subjects and four patients with severe burns. ImAA was found to be normal constituent of human urine. In the cases of burn, where the histamine excretion was increased during the first days after the accident, the ImAA excretion was normal and showed no significant variations. Administration of histamine or histidine gave a slight increase in the excretion of ImAA.

The catabolic pathways of histamine in vivo have, in the last few years, been clarified, particularly by the works of Schayer (1956) and Tabor (1956). The results have recently been reviewed by Schayer (1959). Studies with C^{14}-labelled histamine in man by Schayer and Cooper (1956) and Nilsson *et al.* (1959) have shown that the principal route in the histamine destruction was methylation to methylhistamine or its oxidation product methylimidazole acetic acid. The latter product constituted about 45 per cent of all C^{14}-labelled compounds found in the urine. A minor part of the histamine was inactivated by histaminase to imidazole acetic acid riboside (20 per cent) and imidazole acetic acid (10 per cent). 2—3 per cent was excreted as free histamine which agrees with earlier results (Adam 1950, Duner and Pernow 1958).

There are no known non-isotopic procedures for the determination of the major histamine metabolites in the urine methylimidazole acetic acid and imidazole acetic acid riboside. Methods for isolation of imidazole acetic acid (ImAA) from urine have been described by HANSON (1957) and BALDRIDGE and TOURTELLOTTE (1958). Both methods employ adsorption on ion exchange resins and identification on paper chromatography by diazo reagents. With this method HANSON (1957) found no ImAA in urine from normal human beings, while large amounts (650 and 2,600 µg per 24 hours) were extracted in two cases of urticaria pigmentosa. These patients are known to excrete large *amounts of free histamine in the urine* (BROOKER *et al.* 1958).

It is known from experiments on rats and dogs, that ImAA is formed also from histidine without histamine being involved (BALDRIDGE and TOURTELLOTTE 1958, LINDELL and SCHAYER 1958). In these animals the urinary excretion of ImAA is therefore no specific measure on the endogenous histamine release. However, since the daily histidine intake in man seems to be rather constant at an ordinary diet (BLOCK and BOLLING 1951), the urinary excretion of ImAA might be supposed to reflect changes in the histamine release. In order to study this hypothesis the effect of administration of histidine and histamine on the ImAA excretion was studied in healthy human subjects. The excretion was also studied in patients with severe burns, showing a high endogenous histamine liberation during the first days after the burn accident (BIRKE *et al.* 1957).

Material and Procedure

The urinary excretion of free and conjugated histamine, histidine and ImAA was studied in four healthy male subjects and three male patients with severe burns (20—40 per cent body area). The normal subjects were fed a standardized diet with a calculated histidine intake of 2—3 g per day.

The urine was collected in 24 hour periods. In the normals control samples were collected during 2—3 days. Then for two days 3 mg histamine base diluted in 1 000 ml saline was given each morning during four hours. After one or two control days 5 g of l-histidine was given by mouth at nine o'clock in the morning.

Analytical methods

Free and conjugated histamine were extracted and assayed as described earlier (DUNÉR and PERNOW 1956). All values refer to histamine base.

Histidine and ImAA. 50 ml urine was adjusted to pH 5 with a saturated solution of sodium carbonate and concentrated to dryness in vacuo at 35—45° C. The residue obtained was dissolved in 5 ml of water. After centrifugation the supernatant was passed through Dowex 1 column (1 × 40 cm) which had previously been washed with 2 N hydrochloric acid and water. The compounds were eluted with 1 N HCl. Fractions of 4 ml per 10 min were collected with an automatic fraction collector. Aliquots of 0.05 ml from fraction 1—10 were placed on filter papers (Whatman 1) and chromatographed in a one-dimensional descending system with butanol : acetic acid : water (40 : 10 : 10) as a solvent. Histidine and ImAA were used as reference substan-

Table I. Normal subjects

Case	Day	Procedure	Urine ml/24 hrs	Histamine µg/24 hrs Free	Histamine µg/24 hrs Conjugated	Imidazole acetic acid mg/24 hrs	Histidine mg/24 hrs
1	1	—	800	6.4	17.6	3.4	131
	2	—	750	8.2	15.5	2.3	64
	3	Histamine 5 mg	1 750	184	—	4.3	100
	4	Histamine 5 mg	2 200	161	—	3.9	145
	5	—	1 100	25	33	3.2	131
	6	Histidine 5 g	1 800	19	28	3.6	198
	7	Histidine 5 g	1 500	15	24	5.0	173
2	1	—	690	13	15	0.8	211
	2	—	700	8.4	9	0.3	276
	3	Histamine 5 mg	1 400	110	45	0.7	312
	4	Histamine 5 mg	1 550	160	81	0.8	366
	5	—	700	50	24	0.6	—
	6	Histidine 5 g	800	7.2	12	1.5	339
	7	—	900	9.0	19	0.9	188
3	1	—	1 000	19	32	1.1	162
	2	—	600	15	21	0.8	—
	3	Histamine 5 mg	1 500	160	83	1.8	145
	4	Histamine 5 mg	1 600	214	121	2.4	165
	5	—	900	26	67	0.5	119
	6	Histidine 5 g	1 300	15	19	4.2	238
	7	Histidine 5 g	1 325	18	23	5.0	178
	8	—	1 100	17	20	0.9	—
4	1	—	600	16	16	1.7	164
	2	—	700	11	6	0.5	134
	3	Histamine 5 mg	1 400	52	72	2.9	183
	4	—	1 000	21	17	0.9	157
	5	Histamine 5 mg	1 500	63	51	—	168
	6	—	600	12	20	0.9	134
	7	—	700	8.5	13	1.1	143
	8	Histidine 5 g	1 100	14	10	19.8	197
	9	Histidine 5 g	1 000	19	21	7.0	226
	10	—	900	17	34	2.6	134

ces. After drying, the chromatograms were sprayed with diazo reagent (sulfanilic acid followed by 10 per cent sodium carbonate solution). The coloured spots of histidine and ImAA were cut out and the colour eluted for one hour in 3 ml of tertiary butanol: water: 10 per cent sodium carbonate solution (25: 25: 5) (HANSON 1957). The colour intensity was read at 500 mµ in Beckman DU spectrophotometer. As standards were used the histidine and ImAA references from the chromatograms treated in the same way.

Preliminary results gave recovery of 83—87 per cent (five experiments) of ImAA added to normal urine.

ImAA was synthesized and kindly put at our disposal by Recip, Stockholm.

Table II Burns

Case	Day after burn accident	Urine ml/24 hrs	Histamine µg/24 hrs		Imidazole acetic acid mg/24 hrs	Histidine mg/24 hrs
			Free	Conjugated		
1	1	750	80	49	2.6	113
		760	59	40	1.2	126
	3	730	16	22	2.9	139
	7	1 120	7.2	15	4.8	70
	8	760	11.5	23	3.4	66
2	1	1 600	58	93	4.8	44
	2	1 600	40	68	1.9	75
	3	1 [illegible]00	9	4	3.1	84
	7	1 970	12	32	5.6	50
	8	2 370	14	21	7.5	95
	9	3 150	11	18	9.1	97
3	2	1 300	64	128	3.4	120
	3	1 320	28	73	4.5	140
	4	970	5	21	2.1	150
	5	1 050	11	24	4.1	270

Results

The results from the healthy subjects are given in Table I and from the burns in Table II.

Healthy subjects. The excretion of free and conjugated histamine during the first control periods varied within 6.4—19 µg per 24 hours and 6—3[illegible] µg per 24 hours respectively. An increase up to 214 µg per 24 hours and 1[illegible]1 µg per 24 hours respectively was obtained during the days of histamine administration. *The increase in excretion of free histamine equalled 1.0—4.3 per cent* of the infused amounts.

There was no significant change in the excretion of histamine during histidine administration when compared to control periods.

The amount of excreted ImAA during the first control periods was 0.3—3.4 mg per 24 hours. During the days of histamine administration the ImAA excretion was 0.7—4.3 mg per 24 hours and during histidine administration *1.3—19.8 mg* per 24 hours.

The histidine excretion during the first *control* periods was *64—276 mg* per 24 hours. During the days of histamine administration the histidine excretion was 100—365 mg per 24 hours and during histidine administration 1.3—339 mg per 24 hours.

Burns. The excretion of free and conjugated histamine during the first day after the burn accident was 58—80 µg per 24 hours and 49—128 µg per 2[illegible]

hours respectively There was a significant decrease in th excretion during the following days.

The excretion of ImAA was 2.6—4 8 mg per 24 hours during the first day after the burn accident and showed no clear tendency to change during the following days.

The excretion of histidine was 44—120 mg per 24 hours during the first day and there was no significant change during the rest of the observation time.

Conclusions

The values obtained for free and conjugated histamine in urine in the normal subjects during the control periods were within the normal ranges earlier reported (DUNÉR and PERNOW 1957) During intravenous infusion of histamine the urinary excretion of free histamine increased significantly The percentage of infused histamine excreted as free histamine was of about the same magnitude as previously reported (ADAM 1950 DUNÉR and PERNOW 1958)

In the burn patients a significantly higher histamine excretion was noticed during the first day after the accident. The histamine values then decreased successively and were normal on the third day These findings are in agreement with earlier observations (BIRKE *et al* 1958)

By the method described it was possible to show that ImAA is a normal constituent of human urine. This is in contrast to earlier report, where ImAA is considered to be present only in cases with increased endogenous histamine release (HANSON 1957) The values obtained for histidine excretion were within earlier reported normal ranges (WOODSON 1948, ULRICH 1954)

The purpose of the present investigation was to determine if the ImAA excretion increased with histamine administration or in conditions of increased histamine release and thus could be used as a measure of the endogenous histamine metabolism. It was shown however that ImAA in the urine increased only slightly after infusion of large amounts of histamine and that conditions with significantly increased endogenous liberation of histamine such as the initial stage of burn gave no raised excretion of ImAA. Even if the number of the examined cases are small the results seems to show that in a single case small or moderate changes in the endogenous histamine release are not significantly reflected in the excreted amount of ImAA. In other conditions, however with even larger urinary excretion of free histamine a high output of ImAA has been observed (HANSON 1957) It was further shown, that the ImAA excretion also varies with the histidine intake, which earlier has been observed in animals (BALDRIDGE and TOURTELLOTTE 1958, LINDELL and SCHAYER 1958) It can therefore be concluded, that determination of ImAA in man can not be used clinically to estimate the endogenous histamine formation.

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Transmission and Reflection of High Explosive Shock Waves in Bone

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Abstract

Clemedson, C. J. and A. Jönsson. *Transmission and reflection of high explosive shock waves in bone.* Acta physiol. scand. 1961. *51.* 47—61. — Various kinds of bone such as femur, ribs with intercostal muscles, sternum with ribs and intercostal muscles and spinal column from ox, and femur and femur + tibia with intact stifle joint from horse were exposed to high explosive air shock waves in a detonation chamber. The patterns of the pressure wave in the bone caused by the shock wave were recorded piezo-electrically by means of a barium titanate pressure transducer. The transmitted wave consists of two parts. The first one, called the front pressure wave, is due entirely to the impact of the air shock wave on the front surface of the object. The second part is due mainly to the progressive loading of the static pressure in the air shock wave as it passes by the object. Due to the inhomogeneity of bone, the transmitted part of the incident shock wave is more or less changed since it is diffused and broken up by reflection and scattering. It is less changed in a long bone such as femur than in e. g. sternum or the spinal column. It is strongly changed by the passage between the two bones in a joint. Strong reflections occur at the bone surface. The velocity of the wave is about 3,500 m/sec. in the long bones. In the spinal column and sternum it is considerably lower and is dependent on the length of path covered.

When a sound wave or a finite pulse such as an elastic disturbance (pressure wave) caused by a high explosive shock wave propagates through a human or animal body, the wave will be more or less modified, since it is diffused and broken up by reflection and scattering due to its passing across a large number of boundary surfaces and regions of different density. This modification of the

incident shock waves in biological tissues is characteristic and represents the essential difference in the quality of shock waves in air and water on the one hand and the elastic disturbances caused by shock waves in the body on the other. The properties of shock waves produced by the impact of a high velocity missile and transmitted through muscle, liver, stomach, intestinal wall and human skull bone and beef ribs have been studied by HARVEY and MCMILLEN (1947) employing the spark shadowgram method. The heterogenous structure of these tissues caused a dispersion of the waves and made them appear as a series of wavelets. In a previous study (CLEMEDSON and PETTERSSON 1956) the pressure patterns in some body regions of rabbits exposed to high explosive blasts were recorded by means of a piezo-electric pressure transducer. The incident shock wave was found to be changed to a different extent in the various regions studied, the most pronounced changes occurring in the thorax.

The velocity of an air shock wave increases rapidly with increase in peak pressure. In water and in biological tissues, due to their low compressibility, the velocity of the shock wave produced elastic disturbance is almost unaffected by an increase in peak pressure. To a very good approximation, the velocity of such a disturbance in water and most biological tissues can be considered to be equal to the velocity of sound in water, i.e. about 1,500 m/sec. It can be assumed, however, that this does not apply to tissues containing air or gas, e.g. the lungs, and to denser tissues such as cartilage and bone.

Since bone is the tissue in the body which has the greatest density, it would seem likely that the skeleton should influence a blast wave passing through the body more than any other structure, possibly with the exception of the lungs. The scope of the present investigation has been to study these problems with special reference to (1) the propagation and attenuation of an elastic disturbance caused by a high explosive shock wave in osseous tissue, and (2) the effects of surrounding soft tissues on the reflection of such a disturbance against bone.

Materials and Methods

The specimens of bone used were: femur, ribs with intercostal muscles (part of the chest wall), sternum with unilaterally attached ribs and intercostal muscles and spinal column from ox and further femur and femur plus tibia with stifle joint from horse. With the exception of the intercostal muscles all musculature was carefully removed from the bone. In the specimen of femur + tibia the muscles were carefully removed in order to keep the stifle joint intact. The specimens were exposed with the long axis of the bone parallel with the direction of propagation of the incident shock wave. The end surface of the bone turned against the charge was ground plane in order that the shock waves always should hit the bone surface strictly perpendicularly. In the case of ribs with intercostal muscles the direction of propagation of the shock wave was across the specimen.

The bone specimens were exposed to the shock waves in a detonation chamber described earlier (CLEMEDSON 1949, CLEMEDSON and CRIBORN 1955). The chamber was open in the distal end (cf. CLEMEDSON and CRIBORN 1955) and therefore the shock wave produced was of short duration (overpressure phase about 3 msec). The charge employed

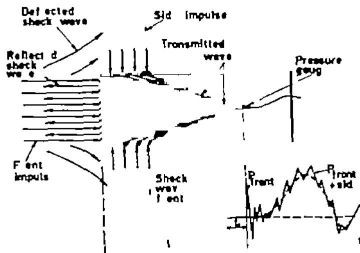


Fig 1 Schematic diagram of phenomena accompanying the impact of high explosive air shock wave on biological medium. In the upper part of the figure is shown the incident deflected and reflected parts of the shock wave and the generation of the transmitted waves. In the lower right part of the figure is shown the corresponding pressure-time diagram showing the two kinds of transmitted waves, e.g. the front pressure wave and the static pressure wave. P_{front} and $P_{front+sid}$ are the peak pressures of these two waves. t_i is the time interval from the impact of the shock wave to the arrival of the front pressure wave.

was plastic high explosive with the chief constituent being pentaerythritol tetranitrate (PETN) The weights of charge used were 2 and 4 g. The charge was fixed on the electric blasting cap (containing 0.2 g lead azide and lead trinitroresorcinate and 0.7 g trinitrotoluene) employed for ignition of the charge. The distance from the charge to the end surface of the bone specimen was 100 cm in all experiments, *i. e.* the plane surface of the bone was 54 cm from the opening of the chamber This distance was used, because at that level of the chamber the shock wave has been found to be subject to minimum reflections and interferences from the walls and the opening At that level in the chamber the peak static overpressure in the air shock wave is 2.4 kg/cm² and 3.8 kg/cm² for 2 g and 4 g charge respectively The maximum reflection overpressure (against a plane, rigid wall perpendicular to the direction of propagation of the shock wave) is 7.9 kg/cm² and 13.2 kg/cm² respectively The velocity of the air shock wave at that level is for the two weights of charge used about 580 m/sec and 680 m/sec, respectively

The pressure transducers employed for recording of the shock wave patterns were the same type of barium titanate pressure transducer Model BC-10, manufactured by Atlantic Research Corp., Alexandria, Va., U. S. A., that have been used in a number of earlier physiological blast studies in our laboratory (CLEMEDSON and PETTERSSON 1956, CLEMEDSON *et al.* 1956, CLEMEDSON 1956, CLEMEDSON and KOLDER 1959) Oscillations in the recording circuit are found which indicate a natural frequency of the gauge of about 210 kc. The capacitances of the gauges with their cables are about 0.007 μF and when connected to the recording instrument, a Tektronix cathode ray oscilloscope 535, having an input resistance of 1 megohm, the time constants varied between 6 and 7 msec. Owing to the complexity of the recorded wave patterns and the relatively short durations of their most interesting parts, it has proved not to be necessary to use special amplifiers in order to increase the time constants.

The transducer was calibrated in air for shock waves passing parallel to its long axis as well as perpendicular to this axis. The tests showed that, for static pressures between 2 and 4 kg/cm² the transducers are in round numbers 15 per cent more sensitive when used with their long axes perpendicular to the direction of propagation of the air shock wave. The orientation of the gauge is, however, of much less importance when used in a medium such as bone tissue. The sensitivities of the gauges used were between 1.15 and 1.35 volts/kg/cm².

To enable the insertion of the pressure transducer into the bone specimen a hole was drilled in the bone. After insertion of the gauge, the hole was filled with molten gelatine which after coagulation assured a good contact between the transducer and the bone.

Results

A schematic representation of the pressure time curve in a biological tissue exposed to a high explosive air shock wave is shown in Fig. 1. When a body of the kind considered here is exposed to the load from an air shock wave the elastic disturbances set up inside the body at some distance from the area of application of the load, due to differences in velocity of propagation of the waves in air and in the body, are split up into two main parts (see Fig. 1). The first part is associated only with the impact of the air shock wave on the front surface of the object and consequently with a reflection pressure. The second part is due mainly to the progressive loading of the static pressure in the air shock wave as it passes by the object. The first part of such a disturbance in the body in question will from now on be called the front pressure wave and the second part the static pressure wave as this latter part in the recording points used is supposed to originate mainly from the load of the static pressure in the air shock wave. The front impulse caused by the incident air shock wave, which is a reflection impulse, is to a high degree dependent on the properties of the exposed medium.

When the air shock wave travels along in the detonation chamber and embraces the tissue specimen, the static pressure wave from the side surfaces successively reaches the pressure gauge delayed due to the lower velocity of the shock wave in air. The static pressure curve is rising only slowly due to the fact that this pressure wave is integrated of a very large number of impulses composed of parts with different path lengths in air and in tissue (see Fig. 1). The effect of the static load (side-on pressure) on a point in the object at some distance from the surface of application of the front impulse load probably reaches its maximum when the distance covered by the side impulse to the point in question is a minimum. Due to the finite length of the object used in this investigation, very complex reflection phenomena are created which influence the pressure wave pattern.

The appearance of the air shock wave generated in the detonation chamber is shown in Fig. 2. The leading part of an air shock wave, i.e. the transition zone in which the first pressure rise takes place is actually very small in thickness, probably of the order of 10^{-5} cm or just around the mean free path of the air

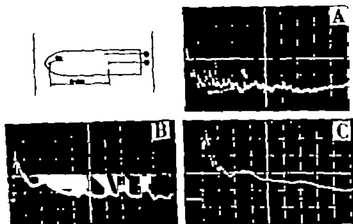


Fig. 2. Recordings of pressure pattern in the incident air shock wave. Weight of charge (Ch) used: A. and B. 2 g, C. 3 g. *Time* (between vertical lines): A. 1 msec, B. 200 μsec, and C. 20 μsec. *Pressure intensity* (between horizontal lines): 0.75 kg/cm² in all three recordings. The upper left picture is a schematic diagram showing the location of the pressure gauges (1 and 2) in the detonation chamber. Gauge 1 is used for synchronization of the sweep start of the oscilloscope with the arrival of the incident air shock wave front at the front surface of the specimen. Gauge 2 is used for recording of pressure pattern within the specimen.

molecules under normal conditions corresponding to a rise time of the pressure of only a small fraction of a microsecond. This property of an air shock wave front can, however, not be expected to be truly reproduced by a pressure gauge of the type used in this study since it has finite dimensions, and it, therefore, takes a certain time for the shock front to sweep over its surfaces (COLE 1948). Furthermore, the arrival of a shock front to the gauge sets up a disturbance within it, causing internal reflections. These phenomena are seen in Fig. 2 C. Due to the above mentioned limitations of the gauge the steep shock front may due to the sweep time used, be reproduced as a sloping line with small oscillations.

The following characters of the disturbances in the biological medium will be discussed, viz. the general wave pattern, the velocity of propagation, and the amplitude of the front pressure wave and of the static pressure wave.

General pressure wave pattern

A typical pressure wave pattern in a long bone (femur of ox) is shown in Fig. 3 A. The first, small peak to the far left in the curve is the front pressure wave and the highest peak in the middle of the curve mainly corresponds to the maximum static pressure. The curve clearly demonstrates the complex pressure wave pattern described above. As the smaller oscillations in the curve have frequencies which are far from the resonance frequency of the gauge, it must be assumed that they mainly are due to reflection phenomena in the bone. It should be pointed out, however, that the reactions of this type of gauge, when introduced in such a medium as bone is not known in detail.

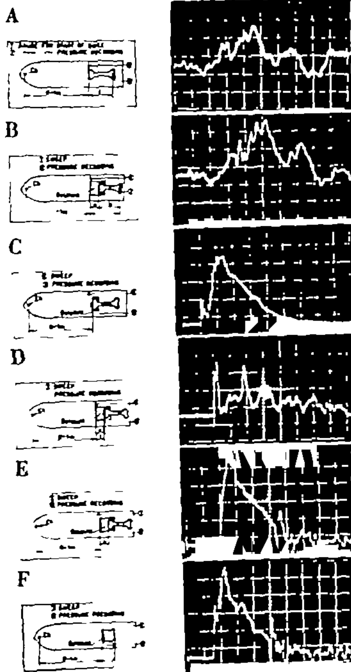


Fig. 3. Recordings of pressure patterns in an os femur. The location of the pressure recording gauge is shown in the schematic diagrams in the left part of the figure. The small letters denote: l the distance covered by the shock wave from front of specimen to recording gauge; a distance from front surface of gelatine body to front surface of bone. Time, pressure sensitivity (between horizontal lines) and distance covered by the pressure wave in specimen are in
A. 200 µsec, 0.4 kg/cm and l 310 mm,
B. 200 µsec, 0.4 kg/cm, 15 mm, b 310 mm, l 325 mm
C. 50 µsec, 1.5 kg/cm
D. 50 µsec, 1.5 kg/cm, l 45 mm, a 55 mm
E. 50 µsec, 1.5 kg/cm, 140 mm and
F. 50 µsec, 1.5 kg/cm, 140 mm.

Fig. 3 B is a similar recording from a bone to the front end of which had been cast a block of 20 per cent gelatine gel in order to simulate a body of soft tissue. The dimensions of the cylindrical block was: length 200 mm, and diam. 100 mm

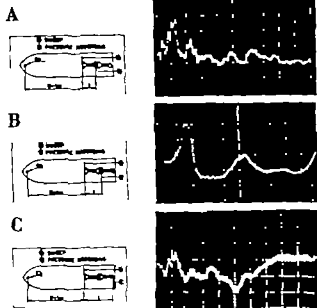


Fig. 4. Recordings of pressure patterns in bone femur + tibia with intact stifle joint. Schematic diagrams on left part of figure show location of pressure gauge. Time, pressure sensitivity and distance covered by pressure wave in specimen are in
A. 1 msec, 0.4 kg/cm
l = 325 mm;
B. 1 msec, 0.4 kg/cm
l = 410 mm; and
C. 2 msec, 0.08 kg/cm^2
l = 720 mm.

About 100 mm of the distal part of the bone was within the gelatine body. A comparison with Fig. 3 A shows that the pressure wave pattern is not significantly altered by a soft medium, which the wave has to pass when being transmitted from air to bone.

The shock wave patterns were essentially the same in the horse bone specimens.

In some experiments the bone marrow in the diaphyses had been removed. This did not significantly change the wave pattern.

A number of experiments have been performed in order to study the reflection of a shock wave against a bone surface. Fig. 3 C is from an experiment, in which the gauge was mounted directly against the flattened end surface of a femur. The disturbances in the rising part of the curve are caused by internal reflections inside the pressure gauge, when the incident shock wave hits the front side of it. The high peak is caused by the impulse reflected against the surface of the bone. The splitting up of the peak in a number of oscillations is in addition to internal reflections, probably also caused by a number of reflections back and forth between the bone and the rear side of the pressure sensitive part of the gauge.

The curve in Fig. 3 D was obtained with the gauge in the same position as in Fig. 3 C, but in this case a gelatine gel body had been cast on to the end of the bone. This will change the pressure pattern considerably. The first high pressure peak is caused by the incident shock wave hitting the gauge. The splitting up of this peak as seen in the previous curve (Fig. 3 C) is now almost quite absent. The second pressure peak appearing about 80 μsec later and having about

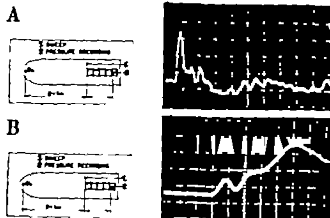


Fig 5 Pressure recordings in a spinal column specimen from ox. Schematic diagrams showing location of pressure gauge in left part of figure. Time pressure sensitivity and distance covered by pressure wave in specimen are in
A. 1 msec 0.4 kg/cm
l = 300 mm and
B 10 μsec, 0.08 kg/cm
l = 110 mm.

the same amplitude as the first peak is caused by the wave that has been reflected back from the bone to the front surface of the gelatine body and from there was again reflected back to the bone surface and the gauge. Since this wave has been reflected against a less dense medium (air) the pressure wave must have undergone a phase shift of 180 and consequently should have appeared as a downward deflection in the curve This downward deflection is, however almost immediately reverted by a new reflection against a denser medium (the bone surface)

When the gauge is located in the front surface of the gelatine body instead of at the surface of the bone curves like the one in Fig 3 E are obtained In this case the second peak, which is the pressure wave reflected back from the surface of the bone is considerably smaller than the first peak. The large first peak in the curve corresponds to the maximum reflection pressure originating from the impact of the air shock wave on the front surface of the gelatine body A comparison with Fig 3 F which is a recording of a shock wave pattern in *a block of gelatine without the bone and in which the reflection of the wave* occurs in the boundary surface to the air instead of against the bone shows that the patterns in the two cases are very much the same

Some experiments were performed in which a specimen of horse femur and tibia with intact stifle joint was exposed to an air shock wave The proximal end of the femur was facing the charge and the pressure wave pattern was recorded at three different levels, re, in the distal end of the femur (Fig 4 A) in the proximal (Fig 4 B) and the distal (Fig 4 C) end of the tibia It is evident, when comparing Figs. 4 A and 4 B that the higher frequencies of the transmitted wave train are strongly damped by the passage through the joint. The completely different wave pattern in Fig 4 C may partly be due to the fact that the recording gauge was located quite near the open end of the detonation chamber and was, therefore probably influenced by reflection and interferences which

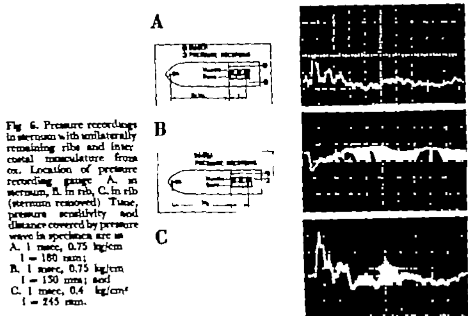


Fig 6. Pressure recordings in sternum with unilaterally remaining ribs and inter costal musculature from ox. Location of pressure recording gauge A. in sternum, B. in rib, C. in rib (sternum removed) Time, pressure sensitivity and distance covered by pressure wave in specimen are as A. 1 msec, 0.75 kg/cm l = 180 mm; B. 1 msec, 0.75 kg/cm l = 130 mm; and C. 1 msec, 0.4 kg/cm² l = 245 mm.

occur when an air shock wave is reflected at the open end of the detonation chamber Consequently the shock wave load will be very complicated at that level.

The character of the pressure wave after passage through a part of the spinal column of an ox is shown in Fig 5 A and 5 B. Fig 5 A shows only the static pressure wave. Due to the low recording sensitivity in this case, the relatively small disturbance caused by the front impulse is not seen in this picture. This is instead shown in Fig. 5 B. The curves contain only few high freqency deflec tions probably due to the damping properties of the spinal column.

A pressure wave transmitted through the sternum is recorded in Fig 6 A. Fig 6 B was obtained from the same specimen, but in this case the gauge had been introduced into one of the ribs attached to the sternum, and the wave had to pass through a number of ribs and costal interspaces before reaching the gauge. The inverted form of the curve as compared with that in Fig 6 A is enigmatic especially as a shock wave passing through a similar specimen, in which the sternum had been removed gave recordings like that in Fig 6 C.

Velocity of pressure waves in bone

One of the main objects of this investigation was to determine the velocity of the front pressure wave in bone caused by a high explosive air shock wave Due to the inhomogeneity of the bone tissue and the varying proportions of compact and spongious bone in different parts of the skeleton, it could be antici pated that the velocity of the transmitted wave should vary within rather wide

Table I Data obtained in blast experiments on femur of ox and horse and femur tibia of horse

Specimen	Number of experiments	Weight of charge in g	Pathlength of shock wave in specimen in mm	Velocity of shock wave in specimen in m sec	Maximum pressure in kg cm² in: Front pressure wave	Static pressure wave
Ox femur	9	2	310	3,960	0.14	0.9
Ox femur	2	2	300	3,200	0.07	0.7
Ox femur	2	4	300	3,130	0.12	1.0
Ox femur	2	2	276	3,640	0.11	0.9
Ox femur	2	4	276	3,710	0.15	1.3
Ox femur with marrow removed	2	2	300	3,300	0.05	0.6
Ox femur with marrow removed	2	4	300	3,190	0.1	1.6
Ox femur with marrow removed	2	2	276	3,840	0.06	1.6
Ox femur with marrow removed	2	4	276	3,[illegible]90	0.1	2.8
Horse femur	6	2	354	3,470	0.01	0.2
Horse femur	4	2	315	3,370	0.07	0.5
Horse femur	2	2	300	3,600	0.02	0.8
Horse femur	2	4	300	3,540	0.03	1.1
Horse femur with marrow removed	2	2	315	3,290	0.01	0.6
Horse femur with marrow removed	2	4	315	3,200	0.05	0.7
Horse femur with marrow removed	2	2	300	3,610	0.015	0.3
Horse femur with marrow removed	2	4	300	3,310	0.02	0.7
Horse femur + tibia	3	2	748	3,190		0.12
Horse femur + tibia	2	4	748	3,300		0.13
Horse femur + tibia	4	2	410	3,100		0.39
Horse femur + tibia	1	4	410	3,110		0.[illegible]
Horse femur + tibia gauge in femur	2	2	325	3,490	0.05	0.[illegible]
Horse femur + tibia gauge in femur	1	4	325	3,320	0.1	1.4

limits. That this is the case is learnt from Tables I and II. In femur and tibia the velocity was found to exceed 3,000 m/sec in all experiments and the mean value obtained is 3,420 m/sec. The distance travelled by the wave within the bone specimen seems to have no influence on the speed of propagation, at least if the length of path does not exceed 750 mm. As seen from Table I, the velocity

Table II. Data obtained in blast experiments on spinal column, sternum and rib + intercostal muscles of ox

Specimen	Number of experiments	Weight of charge in g	Pathlength of shock wave in specimen in mm	Velocity of shock wave in specimen in m/sec	Maximum pressure in kg/cm² in	
					Front pressure wave	Static pressure wave
Spinal column of ox	3	2	312	2,230		2.1
Spinal column of ox	2	2	206	2,640	–	2.1
Spinal column of ox	2	2	108	3,240	0.06	2.4
Spinal column of ox	2	2	86	2,790		1.9
Spinal column of ox	2	2	53	2,870	0.18	2.7
Spinal column of ox	2	2	26	2,830	0.39	2.9
Spinal column of ox	2	4	312	2,100		3.1
Spinal column of ox	2	4	206	2,340		3.0
Spinal column of ox		4	86	2,400		3.6
Ox sternum	4	2	240	1,610	0.045	1.2
Ox sternum	2	2	180	1,900	0.28	1.3
Ox sternum	1	2	110	2,340	0.45	1.0
Ox sternum	1	2	47	3,360	0.8	1.7
Ox rib + intercostal muscles	3	2	200	(700)		0.9
Ox rib + intercostal muscles	3	2	130	(730)		1.0
Ox rib + intercostal muscles	2	2	67	(1,320)		1.0

These low values are essentially derived from the velocity of the air shock wave.

of the pressure wave produced by a 4 g charge does not significantly differ from that of a wave produced by a 2 g charge, when the path covered is the same. The transmission of the wave seems to be mediated essentially through the compacta of the diaphyses, and removal of the bone marrow in the diaphysis does not influence the velocity of the pressure wave. In the spinal column, the mean velocity of the front pressure wave was 2,620 m/sec. In this case there was a tendency towards higher speeds of propagation with diminishing path length. In the sternum this tendency was strongly pronounced. In the specimen consisting of ribs with intercostal muscles very low velocities were found. Evidently the first deflection recorded in these curves is not the front pressure wave but instead the static pressure wave. At least the two first velocity values in the rib + intercostal muscles specimen in Table II are essentially derived from the incident air shock wave and the deflection used for calculation of the velocity consequently is the static wave and not the front pressure wave, which has probably been too small to be recorded. This is due to the fact that the exposed front surface of the end area of the spinal column is too small and that the inhomogeneous structure of the column exerts a rather strong damping effect.

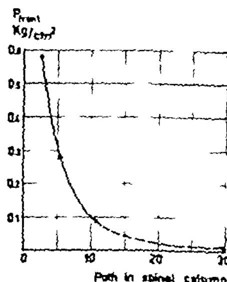


Fig 7 Diagram showing the peak pressure in the front pressure wave as function of the distance covered by the w in a spinal column specimen from ox.

Pressure measurements in the transmitted waves

The maximum pressures in the front pressure wave and in the static pressure wave are found in Tables I and II The maximum pressure in the static pressure wave was found to be 5 to 20 times larger than that in the front pressure wave in the long bones, and up to 40 times larger in the spinal column. The amplitude of the front pressure wave at various levels in the spinal column was especially tudied in one series of experiments. As seen n Fig 7 it diminishes with increasing length of path covered essentially due to the damping properties of the inhomogeneity of the spinal column.

Some experiments were made in order to try to detect an increase in pressure due to reflection against the bone surface Specimens of 20 per cent gelatine

Table III Location of pressure transducer in reflection experiments

Experimental set up according to Fig	Number of experiments	Path length in gelatine block in mm	Maximum pressure in first positive pressure peak in kg cm
3 E, but gauge 140 mm from front surface	6	130 – 140	3 3
3 F	9	130 – 135	6 0
3 E, but gauge 65 mm from front surface	3	60 6	3 6
3 F	10	40 60	5 3
3 E	5	130 – 140	9 5
3 D	3	130 140	4 2
3 C	3	0	4

gel only and of gelatine gel cast on the proximal end of ox femurs were exposed to shock waves. The locations of the pressure transducer in the various experiments are shown in Table III and Fig 3 C—3 F The weight of charge used was 2 g in all experiments. The means of the pressure values obtained in these experiments are found in Table III It is seen that the pressure in the gelatine body is increased by 50 to 100 per cent due to reflection against the surface of the bone. When the pressure gauge is located in the front surface of the gelatine body (Fig 3 F) instead of at the front surface of the bone (Fig 3 D) no such increase in pressure is obtained.

Discussion

The present investigation has shown that the elastic disturbance caused by the incident air shock wave is heavily influenced and changed when passing through various types of bone. When the incident shock wave hits the bone surface — the same also applies for other kinds of tissue — a pressure wave corresponding to the front impulse is propagated through the bone. When the air shock wave embraces the bone this furthermore experiences a static impulse, which is propagated to an arbitrary point within the bone as a very complex train of waves, which are integrated to a more long-lasting static pressure wave. The front pressure amplitude is rapidly damped and at some distance from the front surface of the specimen the amplitude of the static pressure wave may be 5 to 20 times larger than that of the front pressure wave. The front pressure wave is very much dependent on the physical properties of the medium through which it is propagated. The amplitude of this wave is also dependent on the size of the front surface of the exposed medium.

The degree of reflection and transmission of a sound wave or a finite pulse passing through various body tissues is dependent on the physical properties of the tissues. One factor of importance is the density of the medium, which multiplied by the velocity of the wave in the medium gives its wave resistance or acoustical impedance. At a boundary surface between two media of different density a part of the wave in the primary medium is reflected, the rest being transmitted to the secondary medium. The greater the difference in acoustical impedance between two adjacent media (*e g* units of tissue) the greater part of the wave will be reflected back into the primary medium at the boundary surface The tissue in the body which has the greatest density and hence the greatest acoustical impedance, is the bone tissue. A complex pattern of reflection and transmission may be expected in the body It has been shown in the present investigation that strong reflections occur at th boundary surfaces between air and tissue (gelatine gel) and against bone surfaces. The strong decrease of pressure in the pressure wave with increasing distance travelled by the shock wave in the spinal column is also an expression of that.

When an elastic disturbance propagates through a tissue, there is a certain loss of energy to the particles of the medium. In liquid, the loss of intensity over a travelled distance (d) is

$$I = I_0 e^{-\ \ d}$$

where I_0 is the incident intensity and α the absorption coefficient of the traversed medium. The insignificant decrease in pressure with increasing length of path in the femur as compared with that in the spinal column seems to indicate that the reduction due to reflection and dispersion plays a greater role than the energy loss due to form and volume changes in the medium in reducing the amplitude of the pressure wave.

A sound wave propagating through a medium travels with a velocity (c_0) which is characteristic for that medium. It is dependent on the density of the medium according to the following equations

in liquids $c_0 = \sqrt{\frac{1}{K\ \ \rho_0}}$

and

in solids: $c_0 = \sqrt{\frac{E}{\rho_0}}$

in which c_0 = sound velocity ρ_0 = density at rest, K = compressibility and F = module of elasticity As shown by these formulas the properties of density and elasticity is of greatest importance for the propagation of the disturbances discussed here.

For a shock wave, things are much more complicated. According to the Rankine-Hugoniot shock wave equations (c f PENNEY and PIKE 1950) the shock front velocity ( ) is

$$= v_0 \left[(p_1 - p_0)\ (v_0 -\ _1) \right]\ /$$

if v_0 and p_0 are volume and pressure on the low pressure side and v_1 and p_1 the corresponding factors on the high pressure side.

If $p_1 \rightarrow p_0$ on will find that $\ \rightarrow c_0$, or in ther words, t infinitesimally low overpressure the wave velocity approaches that of sound.

FRUCHT has determined the velocity of sound in various soft body tissues. In fat tissue the velocity was found to be about 1 450 m/sec, in brain and kidney about 1,560 m/sec, and in spleen, liver and muscle about 1 580 m/sec. The velocity of a high explosive pressure wave in muscular tissue has been found to be of the same order of magnitude (CLEMEDSON and JÖNSSON) It has been demonstrated in the present investigation that the velocity of pressure waves in long bones is about 3,500 m/sec within rather wide limits. In the spinal column the velocity is considerably lower It is evident that the lower velocity is due to the composition of the spinal column with alternating bone tissue and softer intervertebral discs. It should also be pointed out in this connection that the velocity of elastic disturbances in bone may differ in various directions of propagation due to structural differences.

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Acta physiol. scand. 1961 51 62—67

From the Department of Physiology and the Department of Alcohol Research, Faculty of Medicine (Karolinska Institutet) Stockholm 60

Observations on the Effect of Ethanol on the Urinary Excretion of Histamine, 5-Hydroxyindole Acetic Acid, Catecholamines and 17 Hydroxycorticosteroids in Man

By

E. S. PERMAN

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Abstract

PERMAN, E. S. *Observations on the effect of ethanol on the urinary excretion of histamine, 5-hydroxyindole acetic acid, catecholamines and 17-hydroxycorticosteroids in man.* Acta physiol. scand. 1961 51 62—67 — In healthy young men the urinary excretion of histamine and 5-hydroxyindole acetic acid did not indicate that ethanol in moderate doses (0.5—0 7 g/kg) releases histamine or serotonin in the organism. This makes it unlikely that flushing produced by moderate ethanol doses is associated with the release of one of these substances. The adrenaline excretion after ethanol (0.3—0.4 g/kg) indicated a modest and short-lasting effect on the adrenal medullary secretion. The significance of this effect is discussed No evidence for a concomitant change in the secretion from the adrenal cortex after ethanol (0.5—0.7 g/kg) was obtained

It is generally recognized that many of the symptoms produced by ethanol in man are due primarily to disturbances of the motor and sensory functions of the central nervous system. There are, however symptoms which are less readily explained by such mechanisms. One of these is the cutaneous vasodilatation seen already after moderate amounts of ethanol, the so-called flush Flushing of the skin is known to occur under conditions which favour a release of histamine or serotonin in the body To find out whether ethanol in

moderate amounts affects the release of these substances, the urinary excretion of histamine and of 5-hydroxyindole acetic acid, the principal breakdown product of serotonin, were studied in man after administration of ethanol in the present investigation. More pronounced changes in the release of histamine and serotonin should be detected in this way

Studies dealing with similar problems have been made by others. DRAGSTEDT *et al.* (1940) reported that perfusion of the guinea pig lung with a solution containing ethanol caused liberation of a histamine-like substance into the perfusate, and they suggested that ethanol stimulates gastric secretion via histamine. On the other hand SCHACHTER (1952) found no release of histamine from cat skin when perfusing with an ethanol solution. TROQUET (1958) found no histamine release from various rat tissues *in vitro* after ethanol. In man HOLLER, NEUGEBAUER and SCHMID (1950) noted a slight increase in blood histamine after small ethanol doses.

An interesting finding by SNOW *et al.* (1955) is that flushing as a sign of serotonin release, could be provoked by small amounts of ethanol in some patients with carcinoid tumours. Recently ROSENFELD (1960) has reported that a narcotic ethanol dose (4.5 g/kg) exerts an appreciable inhibitory effect on the metabolism of injected serotonin in mice. In the same report he also mentions that the urinary 5-hydroxyindole acetic acid excretion was decreased in human subjects during a 5-hour period following ingestion of 1—2 g/kg ethanol, but does not give any further data about these experiments.

Recent studies on the urinary catecholamine excretion in man (PERMAN 1958, ABELIN HERREN and BERLI 1958) have indicated that increased secretion from the adrenal medulla occurs after moderate amounts of ethanol (< 1 g/kg) The magnitude and duration of this effect was also studied in the present investigation. Urinary 17-hydroxycorticosteroids have also been measured in similar experiments in order to study any concomitant change in the secretion from the adrenal cortex. It has been shown by several workers (SMITH 1950 1951 FORBES and DUNCAN 1951 SANTISTEBAN and SWINYARD 1956, and others) that in various animals severe, acute ethanol intoxication (3—9 g/kg) is associated with an increase in the activity of the adrenal cortex. KRUSIUS, VARTIA and FORSANDER (1958) did, however not find any significant change in plasma 17-hydroxycorticosteroids or in the eosinophile count in man after a moderate dose of ethanol (< 1 g/kg)

Material and Methods

Seven healthy young men, all with history of very modest ethanol consumption, served as test subjects. The experiments were started at 09.00 after light morning meal. No alcohol intake was permitted during 24 hours preceding the experiment. Smoking was not permitted during the experiment. The ethanol was given as whisky (43 per cent by volume) with the intake spaced over period of 20 min. Urine was collected in periods of 2 or 3 hours.

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Observations on the Effect of Ethanol on the Urinary Excretion of Histamine, 5-Hydroxyindole Acetic Acid, Catecholamines and 17 Hydroxycorticosteroids in Man

By

E. S. Perman

Received 22 August 1960

Abstract

Perman E. S. *Observations on the effect of ethanol on the urinary excretion of histamine, 5-hydroxyindole acetic acid, catecholamines and 17-hydroxycorticosteroids in man.* Acta physiol. scand. 1961. 51. 62—67. — In healthy young men the urinary excretion of histamine and 5-hydroxyindole acetic acid did not indicate that ethanol in moderate doses (0.5—0.7 g/kg) releases histamine or serotonin in the organism. This makes it unlikely that flushing produced by moderate ethanol doses is associated with the release of one of these substances. The adrenaline excretion after ethanol (0.3—0.4 g/kg) indicated a modest and short-lasting effect on the adrenal medullary secretion. The significance of this effect is discussed. No evidence for a concomitant change in the secretion from the adrenal cortex after ethanol (0.5—0.7 g/kg) was obtained.

It is generally recognized that many of the symptoms produced by ethanol in man are due primarily to disturbances of the motor and sensory functions of the central nervous system. There are, however, symptoms which are less readily explained by such mechanisms. One of these is the cutaneous vasodilatation seen already after moderate amounts of ethanol, the so-called "flush". Flushing of the skin is known to occur under conditions which favour a release of histamine or serotonin in the body. To find out whether ethanol in

Table II Urinary excretion of adrenaline and noradrenaline before and after administration of ethanol (0.3—0.4 g/kg) and in control experiment. Ethanol is given at 11.00—11.20 Figures are mean values in group of 3 subjects

	Adrenaline ng/min			Noradrenaline ng/min		
	09.00—11.00	11.00—13.00	13.00—15.00	09.00—11.00	11.00—13.00	13.00—15.00
Control experiment	8.9	8.4	5.8	23	29	25
Ethanol experiment	8.3	14	5.8	23	26	34

Results

Table I shows the diuresis and the urinary excretion of histamine, 5-hydroxy-indole acetic acid and 17-hydroxycorticosteroids in the ethanol and control experiments. The urinary excretion of the substances is not consistently changed by ethanol administration and seems to be independent of the diuresis level. The well-known effect of ethanol on the diuresis is marked in some of the subjects. (The diuresis during the first period, 09.00—11.00 was similar in the two experiments.)

In table II mean values from the catecholamine experiments are given. In these experiments the peak blood ethanol levels were 0.036—0.046 per cent The adrenaline output increased only in the first 2 hour period after ethanol administration. No concomitant effect on the noradrenaline output was observed

Discussion

The results seem to permit the conclusion that moderate ethanol intake causes no major changes in the endogenous release of histamine or serotonin. This makes it unlikely that flushing or other ethanol effects are produced by such mechanisms.

Increased adrenaline excretion was found after moderate ethanol doses. This is in general agreement with previous results obtained in man (PERMAN 1958, ABELIN *et al.* 1958) which have suggested that ethanol increases the secretion from the adrenal medulla. The present results indicate that the increased adrenaline release caused by ethanol is of moderate magnitude and short duration. The lack of effect on the other biogenic amines studied here makes it unlikely that this effect is unspecific. The excretion pattern of catecholamines is of the same type as that seen in hypoglycemia (EULER and LUFT 1952) However FORSANDER, VARTIA and KRUSIUS (1958) and others have found no blood sugar fall in man during the first 2 hours after a similar ethanol dose (< 1 g/kg) In the anaesthetized cat, where ethanol also provokes an increased adrenal medullary secretion (PERMAN 1960) no consistent changes

in the blood sugar were noted within 1 hour after ethanol in doses of < 2 g/kg (PERMAN unpublished observations) Therefore it seems unlikely that the adrenaline release after ethanol serves the glycemic homeostasis. It does, however seem likely that ethanol increases the adrenaline secretion via nervous pathways and that the effect is short lasting and of moderate magnitude, but its pharmacodynamic significance is not clear at present. Possibly the increased adrenal medullary secretion is associated with the general stimulating action noted in man after small amounts of ethanol. Ethanol is considered to depress the inhibitory functions of the cerebral cortex early Results of electrical stimulation in the orbital cortex suggest that this area normally inhibits the secretion from the adrenal medulla via the hypothalamus (EULER and FOLKOW 1958) Ethanol, acting on the cortex, might relieve hypothalamic centers from this inhibiting influence, thus causing increased adrenal medullary secretion. The urinary excretion of 17-hydroxycorticosteroids was mainly unchanged after ethanol which suggests that the adrenal cortex is not concomitantly affected Thus is in agreement with the results of KRUSIUS *et al.* (1958)

The author expresses his thanks to Dr H Duner and Dr B. Pernow who performed the histamine and 5-hydroxyindole acetic acid determinations at the King Gustav V Research Institut Stockholm, and to Dr B. Hökfelt Department of Endocrinology Karolinska Sjukhuset Stockholm, for the 17-hydroxycorticosteroid determinations.

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Effect of Ethanol and Hydration on the Urinary Excretion of Adrenaline and Noradrenaline and on the Blood Sugar of Rats

By

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Abstract

PERMAN, E. S. *Effect of ethanol and hydration on the urinary excretion of adrenaline and noradrenaline and on the blood sugar of rats.* Acta physiol. scand. 1961. *51.* 68—74. — Evidence that ethanol increases the adrenal medullary secretion has previously been obtained from studies of the urinary catecholamine output in man and other studies. — In rats moderate ethanol doses (2.4—2.7 g/kg) increased diuresis and noradrenaline excretion in comparison with similarly hydrated controls (6.1—6.8 ml/kg). However, further data suggest that ethanol effects on the catecholamine excretion in the dose-range 0.7—3 g/kg are explained by the diuretic action of ethanol. Hydration experiments showed that the noradrenaline excretion increased with rising diuresis. The adrenaline excretion was increased at moderate hydration levels. — Ethanol doses above 4 g/kg produced increased adrenaline output and hyperglycemia, as reported by others, and concomitantly caused major changes in the general condition of the rat. It is suggested that the effect of high ethanol doses on the adrenal medullary secretion is secondary to anoxia, due to hypoventilation.

In dogs with acute sublethal ethanol intoxication KLINGMAN and GOODALL (1957) found increased urinary excretion of catecholamines. In other reports (KLINGMAN and HAAG 1958, KLINGMAN, BANE and HAAG 1958, KLINGMAN, HAAG and BANE 1959) strong indirect evidence was presented that ethanol in lethal or sublethal doses markedly increases the activity of the sympathetic nervous system, including the adrenal *medulla*. PERMAN (1960 a) found increased amounts of catecholamines in the adrenal vein blood of anesthetized cats given ethanol.

In man studies on the urinary catecholamine output have indicated that moderate ethanol doses increase the adrenal medullary secretion (PERMAN 1958, ABELIN, HERREN and BERLI 1958). This effect is probably short-lasting and of small magnitude (PERMAN 1960 b). The urinary catecholamine output after ethanol, mainly in a low dose-range, was studied in rats in the present investigation. Previously CRAWFORD and LAW (1958) and others have used the adrenaline and noradrenaline excretion of the rat as indicator of alterations in the activity within the sympathetic nervous system induced by various drugs. Recently WARTBURG, BERLI and AEBI (1960) have reported that an acute ethanol dose (1.2 g/kg) increases the urinary excretion of adrenaline and noradrenaline in rats during a 24-hour period, and that chronic ethanol administration decreases the catecholamine excretion.

Ethanol administration involves administration of considerable fluid volume, and ethanol has in itself a marked diuretic action. It was therefore necessary to use hydrated rats as controls in the present investigation, and also to study the catecholamine excretion in rats hydrated to different degrees to see if the excretion is influenced hereby.

The effects of ethanol on the blood sugar in various species have been reviewed by TENNENT (1941). It has been found that ethanol doses above 3 g/kg generally cause hyperglycemia, whereas lower doses are without effect on the blood sugar. KLINGMAN and co-workers (KLINGMAN and BANE 1955, KLINGMAN, HAAG and BANE 1958) presented evidence that this hyperglycemia is secondary to an increased secretion from the adrenal medulla. The effect of low and high ethanol doses on the blood sugar and on the general condition of the rats was also studied in the present investigation. In man the blood sugar is mainly unaffected or decreased by moderate ($<$ 1 g/kg) ethanol doses (FORSANDER, VARTIA and KRUSIUS 1958, and others).

Material and methods

Rats of the Sprague Dawley strain, body weight 230—350 g, were used in all experiments. They were maintained on a standard stock diet between experiments.

The experiments were performed between 09.00 and 15.00. Groups of 5 rats were used in the urinary excretion experiments. Each animal in the group received the same amount of fluid and ethanol. The dose was calculated from the total group weight, which gives only a minor error as the individual weights varied little within each group. After administration by stomach tube the animals were kept in metabolic cages for a period of five hours without access to food and water. It had previously been established that the diuresis produced by ethanol or by hydration occurs within 3—4 hours after administration and that the diuresis level is almost back to the resting level after 5 hours. The urine was collected in cylinders containing 2-N H_2SO_4 for a final pH of 2—3, and did not come in contact with metal in the collection system as this would influence the catecholamine recovery. After filtration on a suction funnel, catecholamines were determined according to the method of EULER and LISHAJKO (1959). Preliminary experiments had shown that in this way satisfactory excretion values could be obtained.

Table I. Effect of hydration on diuresis and urinary excretion of adrenaline and noradrenaline in rats. The statistical significance of the increase in mean values with increasing hydration, when compared to the lowest hydration level (6.4—7.6 ml/kg) is expressed with the following symbols:

$p > 0.05$
$0.05 > p > 0.01$
$^{++}$; $0.01 > p > 0.001$
$^{+++}$ $0.001 > p$
M. ± S. E. = mean ± standard error

Number of experiments	Hydration ml/kg	Diuresis ml/kg/hour M. ± S. E.	Adrenaline ng/kg/hour M. ± S. E.	Noradrenaline ng/kg/hour M. ± S. E.
19	6.4— 7.6	1.3 ± 0.07	17 ± 2.3	51 ± 3.2
25	12.8—15.0	2.2 ± 0.08^{+++}	30 ± 2.2	73 ± 2.8^{+++}
12	18.3—21.6	3.1 ± 0.12^{+++}	32 ± 3.2	72 ± 4.3^{+++}
10	22.5—25.5	4.2 ± 0.16 $^{++}$	20 ± 2.5	76 ± 5.7^{+++}
4	31.0—31.6	5.5 ± 0.40^{+++}	14 ± 2.0	81 ± 10 $^{++}$

Table II. Effect of one ethanol dose (2.4—3.0 g/kg) at 2 different hydration levels on diuresis and urinary excretion of adrenaline and noradrenaline in rats. The statistical significance of the increase in mean values after ethanol in the 2 groups is expressed with the following symbols.

$p > 0.05$
$0.01 > p > 0.001$
M. ± S. E. = mean ± standard error

Number of experiments	Hydration ml/kg	Ethanol g/kg	Diuresis ml/kg/hour M. ± S. E.	Adrenaline ng/kg/hour M. ± S. E.	Noradrenaline ng/kg/hour M. ± S. E.
19	6.4— 7.6	0	1.3 ± 0.07	17 ± 23	51 ± 3.2
8	6.1— 6.8	2.4—2.7	1.9 ± 0.18	22 ± 4.9	92 ± 11^{++}
25	12.8—15.0	0	2.2 ± 0.08	30 ± 2.2	73 ± 2.8
5	12.9—15.0	2.6—3.0	2.7 ± 0.27*	23 ± 6.7	87 ± 10*

Table III. Effect of ethanol (0.7—3.0 g/kg) on the urinary excretion of adrenaline and noradrenaline in rats compared to hydrated control group

M. ± S. E. = mean ± standard error

Number of experiments	Ethanol g/kg	Diuresis ml/kg/hour M. ± S. E.	Adrenaline ng/kg/hour M. ± S. E.	Noradrenaline ng/kg/hour M. ± S. E.
25 (hydrated controls)	0	2.2 ± 0.08	30 ± 2.2	73 ± 2.8
5	0.7	1.9 ± 0.19	33 ± 4.9	98 ± 6.7
12	1.3—1.4	2.1 ± 0.17	22 ± 4.0	71 ± 5.5
13	2.4—3.0	2.2 ± 0.19	26 ± 4.7	92 ± 7.8

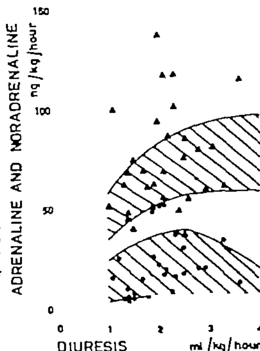


Fig. 1 Urinary excretion of adrenaline and noradrenaline related to *diuresis* in 30 ethanol experiments (0.7—3.0 g/kg). The range of values obtained in hydration experiments without ethanol are denoted by striped areas

○ = adrenaline
▲ = noradrenaline

I the blood sugar experiments all animals received the same amount of fluid, 25 ml/kg. Blood samples were taken from the tip of the tail and the determination method of *Hagedorn and Jensen* was used. The general condition of the animals was roughly assessed by inspection and by handling

Tap water at room temperature was used in the hydration experiments. Ethanol solutions were made up with tap water Dilutions of 5, 10 20, 30 and 40 (v/v) were used, the 40 % solution when doses above 3 g/kg were administered. When lower doses were given, different dilutions were used t each dose level.

Results

The results of the urinary excretion experiments are found in Table I—III Table I shows that when the diuresis was increased by hydration, the urinary *noradrenaline* excretion rose to a higher level and remained elevated with a tendency to increase further with increasing diuresis. The adrenaline output was somewhat increased at moderate hydration levels (12.6—15 0 ml/kg)

Table II shows that ethanol, 2.4—2.7 g/kg produced increased diuresis and noradrenaline excretion when given with a moderate fluid volume (6 1—6.8 ml/kg) and compared with controls receiving a corresponding water volume. However the same ethanol dose had no effect on the noradrenaline excretion if diluted to a larger volume (12.9—15 ml kg) and compared with similarly

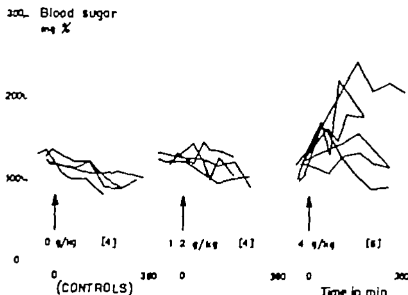


Fig 2. Effect of ethanol on the blood sugar of rats. Figures within brackets denote number of experiments at each dose-level.

hydrated controls. In the latter experiment diuresis levels in the 2 groups did not differ significantly. These findings suggest that here the ethanol effect on the urinary catecholamine output is due to the diuresis produced by ethanol. Table III shows that 5 ethanol doses in the range 0 7—3 g/kg have no significant effect on the urinary catecholamine excretion when compared to controls in a similar diuresis range. Fig 1 shows the catecholamine excretion related to the diuresis in the individual ethanol (0 7—3.0 g/kg) experiments in comparison to the range of values obtained in control experiments with similar diuresis induced by hydration. The rise in noradrenaline output with increasing diuresis can be seen and also that the ethanol experiments do not differ consistently from the control experiments.

In 2 experiments with ethanol in doses of 4 and 5 7 g/kg the adrenaline excretion was strongly increased (80 and 130 ng/kg /hour) whereas the noradrenaline excretion was in the normal range (< 100 ng/kg/hour)

The blood sugar (Fig 2) was mainly unaffected by ethanol in a low dose range (1—2 g/kg) while a dose of 4 g/kg produced hyperglycemia. The general condition of the rats in these experiments was not affected by hydration or by ethanol doses below 3 g/kg. With doses above 4 g/kg gross signs of ethanol intoxication with varying degree of anesthesia regularly appeared. As judged by the colour of the blood samples taken for sugar determination, the animals were in a state of hypoxia at these dose levels. A dose of 6 g/kg was lethal for some of the rats.

Discussion

On the basis of experimental data on the urinary catecholamine excretion in man under various conditions it has generally been assumed that the diuresis can vary over a considerable range without influencing the catecholamine excretion. PITKÄNEN (1956) in a systematic study of the urinary adrenaline output of rats under various conditions, reported that it was largely independent of the diuresis induced by hydration. The catecholamine output at different diuresis levels has apparently not been studied further

The present results, obtained in rats, show that the noradrenaline output increases with a rise in the diuresis level, although the changes noted were small. The fact that increased diuresis induced by ethanol or by hydration gave similar changes seems to rule out an effect of hydration in itself, because ethanol did not affect the catecholamine output. Under the same conditions the adrenaline output was increased at moderate hydration levels only. The reason for the different behaviour of the two catecholamines is not clear at present. That the diuretic effect of ethanol did not always appear is not surprising because "ethanol diuresis" and "water diuresis" are produced by the same mechanism, namely inhibition of the release of antidiuretic hormone (VAN DYKE and AMES 1951 and others). If therefore the amount of fluid administered causes a strong "water diuresis" ethanol does not produce an additional effect (Table II)

Ethanol in the dose-range 0.7—3 0 g/kg did neither overtly affect the general condition, nor the blood sugar level or catecholamine output of the rats. Thus, no evidence for an effect of moderate ethanol doses on the adrenal medullary secretion, as noted in man under similar conditions, was obtained in the rat. As the effect in man is probably moderate and short lasting it is possible that the 5-hour collection period used here did not permit its detection, but marked changes in the output can be excluded. Ethanol doses above 4 g/kg produced increased adrenaline output and hyperglycemia, which is in agreement with the findings made by previous workers. The fact that these effects appear at dose levels where ethanol greatly affects the general condition of the rat suggests the possibility that they are secondary to anoxia which is known to produce similar changes via a strong stimulation of the sympathetic nervous system. That ethanol, as well as other substances with narcotic properties, produces a respiratory depression in a high dose-range is well known from clinical experience. KLINGMAN and HAAG (1958) using the respiratory rate as indicator of the respiratory function, noted that the primary cause of death in ethanol intoxication was respiratory failure in approximately 65 % of their dog experiments.

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Effect of Reserpine on the Storage of Catechol Amines in Brain and Other Tissues

By

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Abstract

BERTLER, Å. *Effect of reserpine on the storage of catechol amines in brain and other tissues.* Acta physiol. scand. 1961 *51* 73—83 — The effect of reserpine on the metabolism of dopamine and noradrenaline in tissues has been studied. Dopamine was found to be depleted from the brain at a higher rate than noradrenaline dopamine decreased to 50 per cent of the normal level in 15 min while the corresponding time interval for noradrenaline was 45 min. The dopamine in the peripheral tissues of the sheep was found to be only slightly lowered within 15 hours, after administration of 2 and 4 mg reserpine per kg whereas the dopamine of the brains were reduced to insignificant amounts. In this respect it behaved in the same way as 5-hydroxytryptamine (5-HT) The rate of disappearance of catechol amines after reserpine is suggested to be dependent on the rate of turnover of the amines. The principal effect of reserpine on the tissue catechol amines and 5-HT is supposed to be due to an interaction of the active transport of the amines into the storage sites. The results of the investigation indicate that the drug does not interfere with the decarboxylation of dihydroxyphenylalanine and 5-hydroxytryptophan.

In 1955 SHORE, SILVER and BRODIE showed that reserpine released 5-hydroxytryptamine (5-HT) from its body stores (see SHORE et al. 1957) A similar effect of the drug on the tissue catechol amines was shown shortly afterwards by CARLSSON and HILLARP (1956) who demonstrated that the drug caused adrenaline to disappear from the rabbit's adrenal medulla. BERTLER, CARLSSON and ROSENGREN (1956) found a complete depletion of noradrenaline from the rabbit's

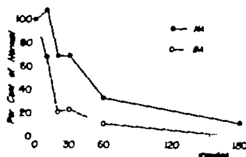


Fig. 1.

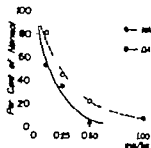


Fig. 2.

Fig. 1. Noradrenaline and dopamine in rabbit brain at various times after reserpine (1 mg per kg body weight)

Fig. 2. Effect of various doses of reserpine on noradrenaline and dopamine in rabbit brain. Time interval 16 hr

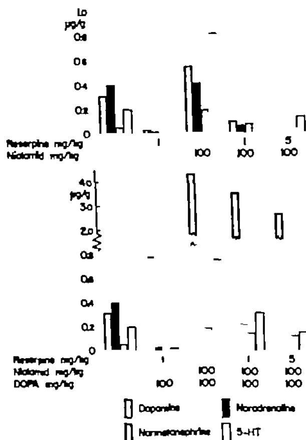
heart and HOLZBAUER and VOGT (1956) observed a marked decrease of the noradrenaline content in the hypothalamus of the cat after administration of reserpine. The data concerning 5-HT indicate that reserpine acts by impairing the storage of this amine (SHORE et al. 1955, CARLSSON, SHORE and BRODIE 1957). The effect of reserpine on catechol amines seems to be an impairment of storage mechanisms, too (CARLSSON et al. 1957), but it is possible that this is not the only effect of the alkaloid.

This paper gives an account of some different lines, which have been tried in order to further elucidate the mechanism of reserpine action. The investigation is not finished, but had advanced so far that the results may be discussed. Of the catechol amines studied, special attention is attached to dopamine (3-hydroxytyramine) for the following reasons: 1) It is less studied than noradrenaline and adrenaline. 2) It offers special interest because of its strict localization to the corpus striatum of the brain. 3) It probably constitutes the precursor of noradrenaline.

Table I. Effect of reserpine on the catechol amines and 5-hydroxytryptamine in various tissues of the sheep

μg/g

	Control			Reserpinized		
	NA	DA	5-HT	NA	DA	5-HT
Corpus striatum	0.1	5.6	0.6	0.0	0.0	0.1
Lung	0.1	3.0	1.6	0.0	1.6	0.6
Heart	2.2	0.2	–	0.0	0.2	–
Spleen	5.7	0.4	–	0.0	0.1	–
Duodenum	0.1	1.5	14	0.0	0.4	3.3



Analysis of 5-HT not performed

Fig. 3. Effect of drugs on noradrenaline, dopamine, normetanephrine and 5-hydroxytryptamine content in rabbit brain.
Time intervals: Reserpine 16 hr; Nialamide 6 hr; DOPA 30 min.

Experimental

Effect of reserpine on dopamine and noradrenaline in rabbit brain. Reserpine in dose of 1 mg per kg body weight was given i.v. to adult male rabbits. The animals were killed by blow on the neck at varying intervals of time after the injection. The brains were immediately dissected out and examined for their catechol amine contents (see below). The results are given in Fig. 1. The effect of varying doses of reserpine on the dopamine and noradrenaline contents in rabbit brain was studied in another set of experiments (Fig. 2).

Effect of reserpine on catechol amine and 5-HT content in various sheep tissues. In order to investigate the effect of reserpine on the dopamine stored in the chromaffin cells of ruminants (FALCK, HILLARP and TORP 1959; BERTLER *et al.* 1959) reserpine in dose of 2 mg and 4 mg per kg was given subcutaneously to tw sheep, weighing about 25 kg. The animals were killed about 13 hours after the injection of reserpine. Two sheep of the same weight served as controls (Table I).

Influence of monoamine oxidase inhibitors and DOPA on catechol amines and 5-HT in rabbit brain. Rabbits were given intravenous injections of the very potent and long-acting monoamine oxidase inhibitor nialamide after the administration of reserpine. The doses and time intervals employed ar seen in Fig 3.

D,L-3 4-dihydroxyphenylalanine (D,L-DOPA) (100 mg per kg) was administered i.v. to rabbits which had previously been treated with reserpine, nialamide or reserpine followed by nialamide. The animals were killed 30 min after the DOPA injection (Fig. 3).

Determinations of catechol amines and 5-HT The determination of noradrenaline and dopamine was performed as described by BERTLER, CARLSSON and ROSENGREN (1958) and CARLSSON and WALDECK (1958) Normetanephrine was determined according to the method described by BERTLER, CARLSSON and ROSENGREN (1959) The application of this method for tissue extracts will be published elsewhere. Estimation of 5-HT was carried out fluorimetrically after purification on a column of cation exchange resin (BERTLER and ROSENGREN 1959) The details of this procedure which have not been published earlier will be given here.

Before the preparation of the column, the resin (AMBERLITE XE-64) has first to be freed from the smallest particles. For this purpose the resin is stirred up in dilute sodium hydroxide solution. The particles which do not settle in 3—5 min are decanted off. This procedure is repeated 15—20 times and then the resin has a suitable mesh-size for preparation of the column (dimension in H -form 6 × 25 mm) The column is washed with 2 N hydrochloric acid, redistilled water and, immediately before use, with 20 ml of N sodium acetate-acetic acid buffer pH 6.5, and a few ml of redistilled water

The tissue 5-HT is extracted with 0.4 N perchloric acid in the same way as described for extraction of catechol amines (see above) The time between homogenization and centrifugation should be as short as possible. If this time interval is extended to mor than 30 minutes the recovery of 5-HT rapidly decreased, probably due to adsorption of the amine to insoluble tissue residues. After centrifugation of the extract at 0° C, the pH of the supernatant is adjusted to about 6.5 by means of 2 N potassium carbonate solution, and the perchlorate formed spun down at 0° C. The extract is then passed through the column. If not more than 5 ml perchloric acid per gram tissue is used in the extraction procedure, 10—12 ml of the extract can be passed through the column without any breaking through. When the extract has passed, the column is washed with 10 ml of a buffer versene solution (0.02 M phosphate buffer pH 6.5, containing 0.2 per cent of the disodium salt of ethylene diamine tetraacetic acid) followed by a few ml redistilled water Elution is performed by means of 3 ml 1.2 N hydrochloric acid. The final determination of 5-HT in the eluate is carried out according to BOGDANSKI et al. (1956) For this purpose 0.8 ml of concentrated hydrochloric acid is added. The fluorescence intensities are read in an Aminco-Bowman spectrophotofluorometer at 316 mμ activating and 565 fluorescent wave-length (uncorrected instrumental values) A UV-filter is pplied in the filter holder in front of the photo cell (CARLSSON and MAGNUSSON 1960) This eliminates the light scatter which otherwise, if high, would interfere with the eadings.

Results

As seen in Fig 1 reserpine (1 mg per kg) caused a depletion of noradrenaline as well as dopamine from the rabbit s brain. The time course of this decrease of the two amines was somewhat different. Dopamine decreased to 50 per cent of its normal value in about 15 min, whereas the corresponding time interval for noradrenaline was about 45 min. The recoveries of the two amines in brain seemed to occur at the same rate which however is not seen in the figure. After

two days about 10 per cent of the normal amount was found On the 9th and 12th about 45 and 65 per cent, respectively had recovered

From Fig 2 it appears that noradrenaline is somewhat more sensitive to low concentrations of reserpine than dopamine.

Reserpine in a dose sufficient to cause a complete disappearance of nor adrenaline from the body stores, caused a comparatively slight lowering of the dopamine content in the peripheral organs of the sheep after 15 hrs (Table I) The dopamine in the corpus striatum, however disappeared completely in that interval of time. Also the amount of 5-HT in peripheral tissues was still remark ably high, whereas the 5-HT in the corpus striatum was reduced to insignificant levels.

Fig 3 gives the results from the experiments with different drug combinations. It is evident that the dopamine level increased after administration of DOPA to rabbits pretreated with reserpine. This increase was facilitated if the animals had been given monoamine oxidase inhibitors before the DOPA injection. Under the latter conditions a significant increase also of the noradrenaline level was observed.

After treatment with monoamine oxidase inhibitors, the 3-0-methylated metabolite of noradrenaline was observed to occur in brain. It is probable that the corresponding metabolite of dopamine, 3-0-methyldopamine, also was increased to some degree. This metabolite is, however not included in the present investigation.

Of particular interest is the demonstration of noradrenaline in brain after administration of a monoamine oxidase inhibitor and DOPA. In most of the previous studies aiming to demonstrate that noradrenaline is formed from dopamine, labelled DOPA or dopamin have been used But, as pointed out by SIMON and WITKOP (1959) the formation of 2 4,5-hydroxyphenyletylamine, with practically the same Rf-value as noradrenaline may invalidat the results obtained with these methods. In this investigation, however noradrenaline was determined by a specific fluorimetrical method. The results strongly support the view that dopamine is the precursor of noradrenaline.

Discussion

Interference with storage mechanism

In order to make the reasoning below clear the metabolism and storage mechanism of the catechol amines has been briefly outlined in Fig 4 This is of course only a hypothetical picture, based on data now available. The various

I three experiments the penetration of L-DOPA into the rabbit brain was compared to that of D-DOPA. When 100 mg L-DOPA per kg body weight was given bout 10 μg of the amino acid was found per g of brain tissue 15 min after the administration. If the same amoun of the D-isomer was given less than 0 5 μg per g of brain could be detected. This observation is in the accordance with the iew that the L-amino acids are transferred into the cells by an activ mechanism (see LEVEVRE 1955)

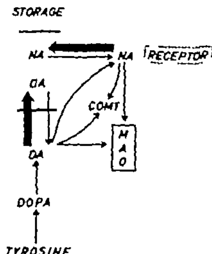


Fig. 4. Hypothetical picture of the metabolism and storage of catechol amines. 1. There seems to be good evidence that the amino acid tyrosine is the precursor of 3,4-dihydroxyphenylalanine (DOPA). 2. DOPA is decarboxylated in the cytoplasm by DOPA decarboxylase. 3. The dopamine (DA) formed is immediately transferred to granules, which probably are of the same type as the adrenaline and noradrenaline (NA) containing particles. In these granules DA can be stored unchanged as in the caudate nucleus. 4. It can, however, also be transformed to NA (e.g. in the hypothalamus). 5. The amines are liberated from their storage sites by nerve impulses and then reach their receptors in relatively high concentrations. 6. Outside the granules a continuous inactivation of the catechol amines is going on by means of monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT).

observations concerning the reserpine effect on the catechol amines (and 5-HT) in tissues may be linked together to comprehend only one mode of action. Reserpine may act primarily by blocking the active transport of catechol amines into the granules. The newly formed dopamine is not taken up by the granules. That this is a possible explanation has appeared from preliminary experiments on rabbit adrenal medulla. Differential centrifugation of extracts of this tissue showed that after injection of DOPA, most of the new formed dopamine was found in the amine containing granules. If the animals were pretreated with reserpine, however, most dopamine was found in the supernatant and only small amounts in the granular fraction, thus indicating an impaired uptake of the amine formed (BERTLER, ROSENGREN and ROSENGREN 1960; BERTLER, HILLARP and ROSENGREN 1960). These experiments are continued. With the aid of the assumption that reserpine acts by blocking the active uptake of catechol amines by the granules the phenomenon discussed in the following section may be easily explained.

Rate of disappearance of monoamines

The rate of disappearance of catechol amines and 5-HT after reserpine administration seems to be correlated to the turn-over of these amines. Dopamine in the brain appears to have a rapid turn-over as demonstrated by CARLSSON et al. (1959) while adrenaline and noradrenaline in the adrenals have a slow one. In accordance with this view the catechol amines in brain disappeared at a much higher rate than those in the adrenal medulla (cf. CARLSSON et al. 1957 b). This also seems to hold for 5-HT. In the intestine, 5-HT has a half-life of several hours and does not disappear from that organ until 16 hours after the administration of reserpine to the animals. The corresponding figures for brain 5-HT in the

rabbit are both about half an hour (UDENFRIEND 1957 BRODIE 1957) The results in Fig 1 may thus be interpreted to indicate, that dopamine has an even more rapid turn-over than noradrenaline. From Table I is evident, that the effect of reserpine on dopamine and 5-HT is very similar

Synthesis of monoamines

The results indicate that reserpine does not affect the formation of catechol amines from DOPA, i. e. it does not lessen the DOPA decarboxylase activity *in vivo*. After pretreatment of the animals with reserpine, a DOPA-injection caused an increase of the dopamine level to about the same degree as if DOPA had been given alone, whereas the noradrenaline level still was very low If the monoamine oxidase had been blocked by nialamid, very high dopamine levels were obtained, and a significant increase of both noradrenaline and normetanephrine as well as 5-HT could be detected. The finding of SHORE et al. (1955) that the excretion in urine of 5-hydroxyindoleacetic acid after a transient increase in reserpinized animals is essentially unchanged seem to rule out the possibility that reserpine interacts with the synthesis of 5-HT The slow accumulation of catechol derivatives after the administration of nialamide to rabbits pretreated with reserpine, on the other hand, might suggest that the formation of DOPA from tyrosine were impaired by reserpine. However the formation of 3 4-dihydroxyphenylacetic acid one of the main products of dopamine degradation, seems to proceed at a normal rate in the caudate nucleus of reserpinized rabbits (ROSENGREN 1960) One possibility to explain this apparent discrepancy would be that the synthesis of catechol amines is not impaired by reserpine only but by the combination of reserpine and nialamide.

It is probable, that the ability of the cells to oxidize tyrosine to DOPA and tryptophan to 5-hydroxytryptophan decides which amine will be stored in the different tissues. Some types of suprarenal medullary tumors are able to synthesize DOPA (STUDNITZ and VERDOALD 1960) Patients suffering from carcinoid-tumors, however do not show an appreciable increase of the principal final metabolite of noradrenaline and adrenaline, i. e. 3-methoxy-4-hydroxymandelic acid. Conversely 5-hydroxyindoleacetic acid seems not to be increased in patients suffering from pheochromocytoma (STUDNITZ 1959 and 1960) Aromatic hydroxylation is therefore probably an important step, which deserves further investigation.

Brain monoamines and behaviour

Nialamide seemed to counteract the effect of reserpine on the behaviour of the animals to some degree. Thus animals which had previously been given reserpine in a dose of 1 mg per kg seemed to become quite normal within a few hours after administration of nialamide. From Fig. 3 it is evident that there was a small increase of catechol amines and a larger increase of 5-HT in the brains of those animals. This definitely argues against the hypothesis of BRODIE and co-

workers that the action of reserpine is due to an increase in free 5-HT It is interesting to note that the rabbits treated with 5 mg reserpine per kg showed much less normalization and also less accumulation of amines. A similar treatment of mice, which had received an even higher dose of reserpine resulted in a slow increase of 5-HT and no alterations in the catechol amine level These animals did not show any restoration of behaviour (CARLSSON LINDQVIST and MAGNUSSON 1960)

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Transfer of Radioactive Iodide Between Mother and Foetus in the Rabbit

By

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Abstract

CRONE, M. and G. WAAGO. *Transfer of radioactive iodide between mother and foetus in the rabbit.* Acta physiol. scand. 1961 51 84—93. — Radioiodide injected subcutaneously into rabbits disappeared more rapidly from th serum of pregnant than non-pregnant animals. To clarify the cause of this difference the ^{131}I uptake in the embryos was measured and higher values were found than corresponded to simple diffusion between mother and foetus. The radioactivity of the foetal gastric juice increased with time. Its ^{131}I concentration was 13 to 109 times higher than in the foetal serum. The ^{131}I concentration in the foetal gastric juice remained high at time when the ^{131}I concentration had decreased to a low level in the foetal serum. This was *considered to indicate that iodide is actively transported through the* foetal gastric mucosa and is accumulated in the foetal gastric juice. Radioiodide was found in a higher concentration in the foetal serum and in an even higher concentration in the foetal part of the placenta than in the maternal serum. A steady state of ^{131}I across the placenta was not obtained as the radioactivity of the maternal serum decreased rapidly A diffusion delay in the foetus was not sufficient to explain the results, which therefore indicated that iodide is actively transported from the mother to the foetus and is concentrated in th foetal part of the placenta. These conclusions were supported by experiments where ^{131}I was administered in such way that the radioactivity of the maternal serum increased or was constant.

Perchlorate was shown to inhibit the ^{131}I uptake in the foetus by inhibiting the active transport across the placenta and through the foetal gastric mucosa. The iodide concentration mechanism of the foetal part of the placenta was also inhibited.

Radioiodide injected into rabbits, guinea-pigs and rats late in pregnancy is transferred to the embryos and is found in a higher concentration in the foetal than in the maternal serum (JOST, MOREL and MAROIS (1952), HIRVONEN and LYBECK (1956), HALL and MYANT (1956)). LOGOTHETOPOULOS and SCOTT (1956) found that there was no organically bound ^{131}I present in the foetal serum, and concluded that active iodide transport by the placental membrane was responsible for the concentration gradient. NATAF, SFEZ, MICHEL and ROCHE (1956, 1957) have investigated the role of the placenta in the transfer of ^{131}I from the mother to the foetus in rats at the end of pregnancy. They found the ^{131}I concentration temporarily higher in the placenta than in the foetal and the maternal serum. This was taken as evidence that the placenta concentrates radioiodide circulating in the mothers blood and thus ensures transport of iodides to the foetus.

These conclusions could only be drawn provided that a steady state of ^{131}I across the placenta was established. In order to obtain this a rather steady level of radioactivity must be maintained in the maternal circulation. The previous workers administered ^{131}I in different ways (i.v., i.m. and s.c.) but the disappearance curve for ^{131}I from the maternal serum was not shown in any case.

Experiments reported in this study showed that ^{131}I injected subcutaneously into pregnant rabbits disappeared rapidly from the maternal serum and no steady state of ^{131}I could be established across the placenta.

This being the case, the finding of higher radioactivity in the placenta and the foetal serum than in the maternal serum did not prove an active transport of iodide from the mother to the foetus or an iodide concentrating mechanism in the placenta. The ^{131}I might have been transferred to the foetus along a concentration gradient early in the experiment when the ^{131}I concentration was high in the maternal serum, and the findings might be due only to a delay in the passage of ^{131}I from the foetus to the mother.

In the experiments reported in this study the disappearance of ^{131}I from the maternal serum was followed, and it was shown that a diffusion delay of ^{131}I in the foetus was not the only explanation of the higher radioactivity in the foetal samples than in the maternal serum.

The discrepancy between the disappearance of ^{131}I from the serum of pregnant and non-pregnant animals was clarified by estimation of the ^{131}I uptake in the embryos. The uptake of radioiodide in the foetus was found to be very high, and radioiodide was shown to accumulate in the foetal stomach.

Experiments were performed with rabbits at the end of pregnancy. A few animals were used on the 20th day of pregnancy at a time when the foetal thyroid is supposed to begin to function (JOST, MOREL and MAROIS 1949).

Finally the effect of perchlorate on the transport of ^{131}I from the mother to the foetus was investigated.

Methods

Animals. Young, white female rabbits weighing 3—3.5 kg were used. The number of embryos is large enough and at the end of pregnancy each foetus is so big that a sufficient amount of blood for the various measurements can be obtained.

Experimental procedure. To investigate the transfer of ^{131}I from the mother to the foetus 50 μC ^{131}I (with less than 2 μg NaI) in physiological saline was injected subcutaneously in the axilla. Blood samples were taken regularly from a marginal ear vein to determine the disappearance of ^{131}I from the maternal serum. At different times (from 1.5 hours to 18 hours) after the injection the rabbits were anaesthetised with intraperitoneal nembutal sodium (40 mg/kg). If necessary additional ether anaesthesia was used. The uterus was opened and each foetus was removed and treated in the following way: Its total radioactivity was measured and the weight recorded. A sample of the foetal blood was taken from the heart. In preliminary experiments radioiodide was found to accumulate in the foetal stomach, and the stomach with its contents was therefore removed. Immediately after the removal of the placenta it was divided into the foetal and the maternal part (HUGGETT and HAMMOND 1952) by blunt dissection. Each part was weighed and homogenised in a Waring Blendor with 15 ml distilled water before the radioactivity was measured.

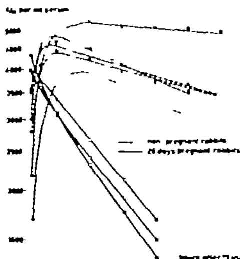
Measurement of radioactivity. The radioactivity of the intact foetus, and in some cases of the stomach and its contents, were measured and related to the injected tracer dose. The radioactivity of the maternal serum, the foetal serum, the foetal gastric juice and homogenates of the foetal and the maternal parts of the placenta were determined after dilution of the samples to equal volumes. A scintillation counter (NaI Tl-crystal, 40 × 25 mm, background 80 c/m) was used. All samples were counted to a standard deviation of less than 2 %.

Only ^{131}I present as iodide was of interest. To correct for possible organically bound ^{131}I analyses of protein-bound ^{131}I (P.B.^{131}I) and ^{131}I bound to amino acids were carried out. Analyses of P.B.^{131}I were done on samples of maternal serum, pooled foetal serum, foetal gastric juice and placenta homogenates. P.B.^{131}I estimations were done in two ways. a) The proteins of the samples were precipitated with 10 (w/v) trichloracetic acid. The P.B.^{131}I was calculated as the difference between the radioactivity of the untreated sample and that of the supernatant solution. b) The samples were dialysed in Visking bags against tap water for 24 hours at room temperature and the radioactivity left in the bags recorded. Analyses of ^{131}I bound to amino acids in placenta and serum were done by paper chromatography (ROCHE, LISSITZKY and MICHEL 1954) after preparation of the samples according to MYANT (1958). Potassium iodide, monoiodothyrosine, diiodothyrosine, triiodothyronine and thyroxine were used as markers. The radioactivity on the chromatograms was measured by mounting them on slide which was moved in front of an end window G-M counter.

Results

Disappearance of ^{131}I from the serum of pregnant and non-pregnant rabbits

Three 28-day pregnant and five non pregnant rabbits were each given 50 μC ^{131}I subcutaneously. The radioactivity of the maternal serum was measured 10 min, 30 min and 1 hour after the injection and thereafter once an hour. ^{131}I disappeared much faster from the serum of pregnant than from the serum of non-pregnant rabbits (Fig. 1). The biological half-life of ^{131}I in serum was 3 hours in pregnant and 12 hours in non-pregnant animals.



The disappearance of ^{131}I from serum after subcutaneous injection into five non-pregnant and three 28-day pregnant rabbits. The radioactivity is plotted against time.

The transfer of radioactive iodide from the mother to the foetus 28 days after mating

Nine rabbits, 28-day pregnant, with a total number of 63 embryos, were each given 50 μC ^{131}I subcutaneously. From 1.5 hours to 18 hours after the injection the embryos and the placentas were removed. The radioactivity of each foetus (per cent of the injected ^{131}I) was recorded and compared with the per cent of the injected ^{131}I that might be expected in the foetus if ^{131}I were equally distributed between the mother and the foetus, i.e. the weight of the foetus in per cent of the mothers weight (the weight of the mother with all the embryos). The mean from each litter is shown in Table I. A significantly higher amount of ^{131}I was found in the foetus than corresponds to simple diffusion. This was studied further by measuring the radioactivity of the maternal serum, the maternal and the foetal part of the placenta, the foetal serum and that of the foetal gastric contents. Table II (rabbits no. 1—9) summarizes the results and shows the maximum radioactivity recorded in the maternal serum. In most experiments this radioactivity of the maternal serum was measured shortly after the ^{131}I injection (Fig. 1).

All the figures in Table II and the following tables represent ^{131}I present as iodide. Less than 2 was protein-bound in foetal as well as in maternal samples except in the two 18 hours experiments, in which less than 5 was protein-bound. There was no evidence of ^{131}I bound to amino acids.

In all experiments the radioactivity at the time of removal of the embryos was higher in the foetal than in the maternal serum (Table II, rabbits no. 1—9). Furthermore the radioactivity was higher in the foetal and the maternal parts of the placenta than in the foetal and maternal serum respectively

Table I The uptake of ^{131}I in the foetus

Rabbit no.	Litter size	Hours after ^{131}I inj	Weight of one foetus[1] (g)	^{131}I in one foetus (per cent of the injected ^{131}I)	Weight of one foetus in per cent of the mother weight	^{131}I in one foetal stomach[1] (per cent of the injected ^{131}I)
28-day pregnant rabbits:						
4	12	1.8	23 1	3.29	0.67	1.62
6	8	4.3	31 4	7 46	1.05	
8	2	18	40.1	7 73	1.23	
9	3	18	29.9	4.90	1 03	
20-day pregnant rabbits:						
13	3	1 7	3.38	0.19	0 10	
14	7	4 4	3.10	0.11	0.06	
28-day pregnant rabbits, after injection of perchlorate:						
15	7	1.5	28.8	0.68	0 82	
16	3	4.3	40	1 10	1.23	
17	9	4.3	29 0	0.82	0.85	0.08

[1] Each figure represents the mean of the litter

Radioiodide was strongly concentrated in the foetal gastric juice. The radioactivity of the foetal serum (excepting the two experiments of 18 hours duration) and of the foetal gastric juice was even higher than the maximum radioactivity of the maternal serum. The radioactivity of the maternal part of the placenta never reached this value.

The transfer of radioactive iodide from the mother to the foetus 20 days after mating

Two rabbits, 20-day pregnant, with a total number of 10 embryos were used. The foetuses and the placentas were removed 1 7 hours and 4 5 hours after the ^{131}I injection. The radioactivity of each foetus was measured and showed slightly higher values than corresponded to equal distribution between the mother and the foetus (Table I) The radioactivity of the different samples are summarized in Table II (rabbits no. 13—14)

1 7 hours after the ^{131}I injection (rabbit no 13) the radioactivity recorded was the same in the foetal and the maternal serum. In all embryos the radioactivity of the foetal part of the placenta was higher than that of the foetal serum, and that of the maternal part of the placenta was lower than that of the maternal serum. The radioactivity of the foetal part of the placenta and in rabbit no 14 that of the foetal serum and the foetal gastric juice was

Table II. The transfer of ^{131}I from the mother to the foetus. The table shows the radioactivity (cpm per ml serum or cpm per g tissue) of maternal serum, maternal placenta, foetal placenta, foetal serum and foetal gastric juice

Rabbit no.	Litter size	Hours after ^{131}I inj.	Max. value of mat. serum	Mat. serum at the time of removal of the foetuses	Maternal placenta	Foetal placenta	Foetal serum	Foetal gastric juice
28-day pregnant rabbits:								
1	9	1.5	4 400	2,280	3,380	5,940	6,190	78,700
2	7	1.5	6,230	2,670			6,300	116,200
3	8	1 7	2,700	2,430	2,540	6,160	5,010	102,600
4	12	1.8	4,370	2,250	2,300	9,500	7 100	106,100
5	9	2.0	2,250	2,000	1,930	3,800	3,150	84,700
6	8	4.3	4,020	1,500	2,000	7 010	6,890	746,000
7	4	4 4	3,770	1,500	2,650	6,120	3,380	404,000
8	2	18	3,040	400	490	2,480	2,210	369,000
9	3	18	4,250	320	520	1,900	1,880	209,000
10		1.2	670	670	410	1,360	980	3,630
11		2.6	850	800	670	1,430	1,030	23,200
12		2 5	630	620	480	1,280	1,340	16,000
20-day pregnant rabbits:								
13	3	1,7	3,900	5,150	2,240	6,890	5,130	3,000
14	7	4.4	3,480	2,230	1,900	5,020	4,500	16,500
28-day pregnant rabbits, after injection of perchlorate:								
15	7	1.5	6,000	5,500	3,290	2,390	2,740	1,860
16	3	4 5	7,300	5,750	3,990	2,920	4,890	5 460
17	9	4 3	4,770	4,350	3,290	2,380	3,380	3,370

Each figure represents the mean of the litter
Propylthiouracil is given before the experiment

higher than the maximum radioactivity of the maternal serum. In principle the results were similar to those found 28 days after mating. The ^{131}I uptake by the foetus increased considerably during the last third of pregnancy. Especially the ability of the foetal stomach to concentrate iodide increased.

Accumulation of ^{131}I by the placenta per se

The ability of the placenta to concentrate radioiodide was furthermore studied in experiments in which the transfer of ^{131}I from the placenta to the foetus was prevented by removing the foetus before the ^{131}I injection.

Table III The concentration of ^{131}I (c/m per g) in placentas with the foetuses removed (II) and in placentas with the foetuses left in utero (I)

Rabbit no.	Litter size	Max. value of mat. serum	Foetal serum	Mat. placenta I	Mat. placenta II	Foet. placenta I	Foet. placenta II
18	5	3,300	14,900	4 470	5,510	27 700	32,300
19	4	3,210	8,670	4 700	7 410	12,800	20,600

Two rabbits, 28-day pregnant, were used. The uterus was opened on the opposite side of the placental attachment. One foetus from each of the uterine horns was removed after ligation of the umbilical cord. Great care was taken not to detach the placenta from the uterine wall. The uterus was closed and the rabbit was given 50 μC ^{131}I subcutaneously. The samples were taken 5.8 hours after the injection. Higher radioactivity was found in the placentas without a foetus than in the placentas with the foetus in utero (Table III).

Experiments with ^{131}I injected into the embryos in one of the uterine horns 28 days after mating

Iodide has been shown to be transferred from the foetus to the mother in rabbits, guinea pigs and rats, (HALL and MYANT (1956), LYBECK and HIRVONEN (1956) and NATAF, SFEZ, MICHEL and ROCHE (1957)). These findings were confirmed by the following experiments. Radioiodide was injected through the uterus and the membranes into the embryos in one uterine horn. Radioiodide was found in the first sample of maternal blood taken 10 min after the injection. The ^{131}I concentration in the maternal serum increased during the first hour after which it remained constant at a level much lower than in the foetal serum (Table IV). This gives a possibility for obtaining a

Table IV The radioactivity (c/m per ml) of maternal serum and foetal serum after injection of ^{131}I in the foetuses in one uterine horn

Rabbit no.	Hours after ^{131}I inj.	Maternal serum 10 min	0.5 h	1 h	1.5 h	2 h	2.5 h	Serum from injected foetuses	Serum from not injected foetuses
10	1.2	20	300	670				7,350	980
11	2.6	100	500	830	850	830	800	4 160	1,030
12	2.5	50	270	470	600	610	620	7,800	1,316

Mean values

rather steady state of ^{131}I across the placenta of the non-injected foetuses. They were removed 1.2 hours and 2.5 hours after the ^{131}I injection. The ^{131}I concentration was higher in the foetal than in the maternal serum and higher in the foetal part of the placenta and in the foetal gastric juice than in the foetal serum. The ^{131}I concentration in the maternal part of the placenta was lower than in the maternal serum. (Table II rabbits no. 10—12.)

The effect of perchlorate on the transfer of iodide from the mother to the foetus

Different anions, particularly perchlorate inhibit the active iodide uptake by the thyroid gland (WYNGAARDEN *et al.* 1952) and the active iodide transport through the gastric mucosa (HALMI *et al.* 1956)

Three rabbits, 28-day pregnant, with a total number of 19 foetuses were used to study the effect of perchlorate on the transport of ^{131}I from the mother to the foetus. 200 mg sodium perchlorate were given i.p. 20 min before the ^{131}I injection and additional 100 mg perchlorate 1.3 hours and 2.8 hours later The rest of the experiments were performed as previously described.

1.5 hours and 4.3 hours after the ^{131}I injection 0.68 % to 1.10 % of th injected ^{131}I was recovered in the foetus (Table I) This was less than should be found in the foetus if ^{131}I was equally distributed between mother and foetus. Comparing these figures with those found in experiments where no perchlorate was given (Table I) it is evident that perchlorate strongly inhibits the ^{131}I uptake in the foetus.

The radioactivity of the different foetal and placental samples was lower than the radioactivity of the maternal serum (Table II rabbits no 15—17)

Discussion

The rapid disappearance of ^{131}I from the maternal serum following subcutaneous injection into rabbits late in pregnancy is mainly due to a very high ^{131}I uptake by the embryos. 50 % or more of the radioiodide in the foetus is localized in the gastric juice. The radioactivity of the foetal gastric juice reaches values of more than 100 times that of the foetal serum (Table II rabbits no. 6, 8, and 9) indicating that iodide is secreted into the foetal gastric juice.

ELMER (1938) mentioned, that iodide was concentrated in the stomach contents in man. Radioiodide injected intravenously in man (HONOUR, MYANT and ROWLANDS 195.) and different adult animals (LOGOTHETOPOULOS and MYANT 1956) was concentrated in the gastric juice. The last authors also reported that ^{131}I was concentrated in the stomach contents in a 50 days old guinea-pig foetus 1 hour after the ^{131}I injection into the mother

The experiments presented in this report show that iodide is secreted increasingly during the last third of the gestation period by the foetal gastric mucosa in rabbit embryos. The radioiodide concentration in the foetal gastric

juice remains high 18 hours after the ^{131}I injection at a time when the ^{131}I concentration is low in the foetal serum (Table II rabbits no. 8—9). This storage of iodide in the foetal gastric juice does not occur in the adult animal. It can be explained by the absence of transport through the intestinal canal during foetal life and slow reabsorption of iodide through the stomach wall.

Following radioiodide injection into 28-day pregnant rabbits the radioactivity of the foetal serum and the foetal and the maternal part of the placenta is higher than that of the maternal serum at the time of removal of the foetus. During the first 4.5 hours after ^{131}I injection the radioactivity of the foetal serum and of the foetal part of the placenta remains higher than the maximum radioactivity recorded in the maternal serum, but the radioactivity of the maternal part of the placenta does not in any case reach this value (Table II rabbits no 1—7). During the same hours the radioactivity of the foetal gastric juice increases significantly. This indicates a net transfer of ^{131}I from the foetal serum to the foetal gastric juice during this period. It is therefore evident that the recording of higher radioactivity in the foetal serum and the foetal part of the placenta than in the maternal serum is not only due to a diffusion delay of ^{131}I in the foetus. It is concluded that iodide is transported from the mother to the foetus against a concentration gradient. Furthermore the radioactivity is higher in the foetal part of the placenta than in the foetal serum indicating, that iodide is accumulated in this part of the placenta. There is no evidence of an accumulation of iodide in the maternal part.

These conclusions are supported by experiments where ^{131}I is injected into the embryos in one uterine horn. In the non-injected foetuses in the other uterine horn higher radioactivity is recorded in the foetal serum and an even higher radioactivity in the foetal part of the placenta than in the maternal serum (Table II rabbits no. 10—12). This can not be due to a diffusion delay of ^{131}I in the foetus or in the placenta as the radioactivity in the maternal serum is increasing during the first hour and then remains constant during the rest of the experiment. There is no evidence of an accumulation of iodide in the maternal part of the placenta.

The ability of the placenta to accumulate iodide is further studied by removing the foetus before the ^{131}I injection leaving the placenta untouched. Higher radioactivity is found in this placenta than in the placenta with intact foetal circulation (Table III).

It must be mentioned that the measurements of the ^{131}I concentration are carried out on the foetal part of the placenta without correction for its content of maternal or foetal blood, both having a lower ^{131}I concentration than the foetal part of the placenta as a whole. The maternal blood constitutes about 35 % of the foetal part of the placenta at the end of pregnancy (MYANT 1958). The foetal blood volume of the foetal part of the placenta is not known. With these corrections in mind it is seen that the accumulation of iodide in the foetal part of the placenta is more pronounced than our figures indicate.

Perchlorate is shown to inhibit the active transport of iodide across the placenta and across the foetal gastric mucosa, and to inhibit the accumulation of iodide in the foetal part of the placenta (Table II rabbits no. 15—17). The mode of action of perchlorate is as yet as unknown as the mechanism of active iodide uptake itself.

We wish to thank C. Thole, mag. scient. for helpful discussion and advice.

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It must be mentioned that the measurements of the ^{131}I concentration are carried out on the foetal part of the placenta without correction for its content of maternal or foetal blood, both having a lower ^{131}I concentration than the foetal part of the placenta as a whole. The maternal blood constitutes about 35 of the foetal part of the placenta at the end of pregnancy (MYATT 1958) The foetal blood volume of the foetal part of the placenta is not known. With these corrections in mind it is seen that the accumulation of iodide in the foetal part of the placenta is more pronounced than our figures indicate



Fig. 1. Excretion of adrenaline (●) and noradrenaline (○) in rats (170—180 g) at +3° C. (———) and +22° C. (— — —). Each point represents the mean of six individual rats.

The large increase in the noradrenaline excretion, and presumably release, on exposure to cold is interesting in view of the strong calorigenic effect of this hormone in cold-acclimated rats as reported recently by HSIEH and CARLSON (1957) and DEPOCAS (1960). The gradual decline in the output may be explained by increasing sensitivity to noradrenaline (cf. DEPOCAS 1960). That noradrenaline is from extra-adrenal origin is shown in adrenalectomized rats. HSIEH, CARLSON and GRAY (1957) have reported that noradrenaline prevents the fall in oxygen consumption caused by hexamethonium in cold-adapted rats. Since there is every reason to assume that noradrenaline derives from the adrenergic nerve endings, it can be inferred that the sympathetic nervous system is involved in the metabolic response to cold-exposure through the release of noradrenaline.

As to the role of adrenaline it seems to represent a second line of defence which need not be called on for great activity unless the environmental conditions become more severe and/or the other mechanism is exhausted.

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From the Department of Pharmacology University of Lund, Lund, Sweden

Occurrence and Localization of Catechol Amines in the Human Brain

By

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Abstract

Bertler, Å., *Occurrence and localization of catechol amines in the human brain.* Acta physiol. scand. 1961. *51* 97—107. — The content of noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) in various structures of the human brain has been investigated. Noradrenaline in rather high concentrations was found in the anterior and middle portion of the hypothalamus, and lower levels were recorded in structures of the mesencephalon and the floor of the 4th ventricle. Dopamine showed a very strict localization to structures which are involved in the extrapyramidal system, i. the basal ganglia and the substantia nigra suggesting that it may be involved in the function of this system. Relatively high concentrations of 5-HT were found in the hypothalamus, the medulla oblongata, the corpus striatum and the thalamus.

The data now available indicate that catechol amines are of importance for the normal brain function. A fact pointing in that direction is the localization of these amines to certain structures of the brain. Thus Vogt (1954) found that the brain regions containing the diencephalic, mesencephalic and bulbar representation of the sympathetic activities also were rich in noradrenaline and adrenaline. Studies on the third catechol amine known to occur in mammalian brain, i. e. dopamine (3-hydroxytyramine) revealed quite a different localization of this substance[1] (Bertler and Rosengren 1959 a and b) high concentrations

[1] The results were first reported Symposium on Catechol Amines, Bethesda, October 16—18, 1958 (Carlsson 1959)

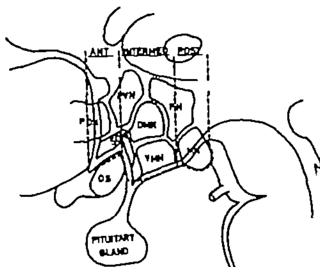
Table I Distribution of noradrenaline and dopamine in the human brain

μg/g

Brain part	Number of estimations	Noradrenaline Range	Mean	Dopamine Range	Mean
Cortex from					
Frontal lobe	2	0.00–0.02	0.01	0.00–0.00	0.00
Anterior central gyrus	2	0.00–0.03	0.02		–0.01
Posterior central gyrus	2	0.02–0.03	0.03	–0.07–(–)0.05	–0.06
Occipital pole	1	–	0.00	–	0.06
Hippocampus + olfact. bulb	1	–	0.03	–	–0.01
Septum pellucidum	1	–	0.03	–	0.04
Basal ganglia					
Caudate nucleus	10	0.00–0.08	0.04	1.50–4.67	3.12
Putamen	3	–0.01–0.05	0.02	3.02–7.72	5.27
Globus pallidus	4	0.00–0.08	0.03	0.11–0.35	0.32
Internal capsule (anterior limb)	1	–	0.00	–	0.42
Claustrum	3	0.00–0.07	0.02	0.16–0.67	0.44
Thalamus					
Anterior nuclei	1	–	0.02	–	0.07
Lateral nuclei	2	0.00–0.07	0.04	0.00–0.02	0.01
Medial nuclei	1	–	0.09	–	0.03
Hypothalamus					
Anterior parts	8	0.34–1.87	0.96	0.02–0.31	0.18
Intermediate part	7	0.24–1.80	1.19	0.03–0.24	0.14
Posterior part	8	0.03–1.01	0.31	0.00–0.39	0.22
Mesencephalon					
Superior colliculus	4	0.07–0.18	0.12	0.09–0.15	0.13
Inferior colliculus	4	0.06–0.33	0.15	0.00–0.15	0.10
Red nucleus	4	0.14–0.27	0.22	0.12–0.35	0.19
Substantia nigra	6	–0.06–0.08	0.04	0.25–0.70	0.40
Basis pedunculi	2	–	0.00	–0.03–0.00	–0.02
Rest	3	0.20–0.29	0.24	0.07–0.15	0.11
Pons	2	0.02–0.05	0.04	–0.01–0.01	0.00
Medulla oblongata					
Dorsal part, containing the floor of 4th ventricle	2	0.10–0.16	0.13	–0.01–0.00	–0.01
Ventral part	2	0.00–0.07	0.03	0.00–0.01	0.01
Cerebellum					
Dentate nucleus	1	–	0.01	–	0.03
Cortex		0.01–0.03	0.02	0.01–0.02	0.07
Pituitary gland	2		0.00	0.01–0.02	0.02

Fig 1 Diagrammatic drawing of the hypothalamic model. The broken lines indicate the boundaries between the subdivisions used in this investigation (see the text).

PON	Pre-optic nucleus
SON	Supra-optic nucleus
PVN	Paraventricular nucleus
DMN	Dorso-medial nucleus
VMN	Ventro-medial nucleus
PN	Posterior nucleus
MN	Mammillary nuclei
OS	Optic chiasm
ANT	Anterior part
INTER MED	Intermediate part
POST	Posterior part



being found n the corpus striatum of the cerebral hemispheres. Preliminary data from this laboratory have shown a similar distribution pattern of this amine in the human brain as in the brains of other mammalian species examined (BERTLER and ROSENGREN 1959 c) This finding was later confirmed by SANO et al. (1960) and by EHRINGER and HORNYKIEWICZ (1960)

In order to get further information about the function of the catechol amines, pecially dopamine, in the central nervous system a more extensive knowledge of the localization of these amines within brain seemed of importance The human brain appeared to be well suited for such an investigation as the various structures can be more readily dissected out. In this paper a detailed account on the occurrence of noradrenaline and dopamine in the human brain is given Some data on the presence of 5-hydroxytryptamine (5-HT) are also presented

M terial and Methods

The brain material was obtained from the utopsy room 1 5—5 hrs after death of the patient. Each piece of tissue was immediately cut into small slices and put into glass ube containing 0 4 N perchloric acid. Estimation of ca echol amines was performed ac ording to he methods developed in this laboratory (BERTLER, CARLSSON and ROSE ...ER 1958, CARLSSON and WALDECK 1958) I order to check the identity of the substances determined, paper chromatography has been performed in few cases. The activ tion and fluorescence spectra of the fluorophores obtained from the brain material were als) compared to those of sy thetical noradrenaline or dopamine The method used for estimation of 5-HT has been briefl outlined by BERTLER and ROSENGREN 1959 A more detailed account of this method will be published elsewhere (BERTLER 1960

Table II Noradrenaline and dopamine in 10 human brains, from which four or more structures
μg/g

	Age of the patient Years 75		52		73		83	
	Hrs after death 2		2		2		2	
	Diagnosis Pulmonary embolism		Heart failure		Mammary cancer		Cerebral arterio-sclerosis	
	NA	DA	NA	DA	NA	DA	NA	DA
Basal ganglia								
Caudate nucleus	0.00	2.80	0.06	3.75			0.05	2.45
Putamen								
Globus pallidus								
Internal capsule								
Claustrum								
[illegible]lamus								
[illegible] part	1.13	0.31	0.78	0.10			0.49	0.12
Intermed. part	1.44	0.13	1.80	0.24			0.24	0.01
Posterior part	0.34	0.28	1.01	0.30			0.17	0.00
Mesencephalon								
Superior colliculus			0.18	0.09	0.16	0.09		
Inferior colliculus			0.33	0.12	0.16	0.06		
Red nucleus			0.22	0.10	0.24	0.19		
Substantia nigra			0.08	0.51	0.07	0.40		
Basis pedunculi					0.00	0.03		
Rest			0.29	0.15	0.23	0.15		

Results

The concentrations of noradrenaline and dopamine in different parts of the human brain are seen in Table I. Most of the dopamine present in the brain was found to be localized to some nuclei of the basal ganglia of the cerebral hemispheres. Thus the caudate nucleus and the putamen contained 3 and 5 μg per g, respectively. The dopamine contents of the globus pallidus and the claustrum were about 10 times smaller. In all the structures of the basal ganglia the concentration of noradrenaline was 0.05 μg per g or less. In the rest of the cerebral hemispheres the concentrations of catechol amines were very small.

have been taken for estimation

Age of the patient Years											
78		76		60		79		87		74	
Hrs after death											
2.5		3		4		4		4		5	
Diagnosis											
Myocardial infarction		Mammary cancer		Leukemia		Circulatory failure		Oesophag cancer		Myocardial infarction	
NA	DA	NA	DA	NA	DA	NA	DA	NA	DA	NA	DA
		0.05	2.80	0.03	4.67					0.03	3.63
		0.02	3.51	0.04	5.54					0.02	3.02
		0.00	0.22	0.00	0.55	0.07	0.11	0.04	0.39		
										0.00	0.42
				0.00	0.67						
				0.59	0.20	0.34	0.21	0.48	0.10		
						0.52	0.21	0.64	0.21		
				0.09	0.11	0.03	0.12	0.23	0.30		
0.06	0.13	0.12	0.15								
0.12	0.12	0.07	0.00								
0.14	0.12									0.14	0.26
0.02	0.44					0.03	0.25			0.03	0.60
0.00	0.00										
0.20	0.07										

Noradrenaline was found in a fairly high amount in the hypothalamus, where relatively small amounts of dopamine, 0.2 μg per g could be detected. Within that structure most of the noradrenaline was recorded in the anterior and intermediate parts. In the former part the preoptic nucleus, most of the supraoptic nucleus and a piece of the paraventricular nucleus were included (Fig. 1) while the intermediate part contained the rest of the paraventricular nucleus, the dorsomedial nucleus and the ventromedial nucleus and part of the posterior nucleus. The posterior part of the hypothalamus was principally made up of the rest of the posterior nucleus and the mammillary nuclei.

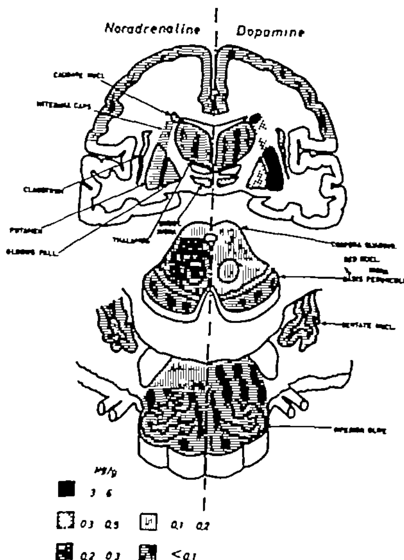


Fig. 2. Noradrenaline and dopamine in the human brain

In the midbrain noradrenaline, in a concentration of more than 0.2 μg per g was found in the red nucleus. A sample containing, among other structures, the mesencephalic part of the reticular formation and the mesencephalic nuclei of the eye, had about the same concentration of noradrenaline. Dopamine was principally concentrated to the substantia nigra where noradrenaline was nearly absent. Significantly lower concentrations of dopamine were recorded in the other parts of the mesencephalon

Table III

1 Brain from 5 months old foetus, taken 4 hrs after death. The sample called "central part" containing the basal ganglia, the thalamus, the hypothalamus and some adjacent structures.

μg g

	Noradrenaline	Dopamine
Central part	0.11	0 07
Mesencephalon + pons	0.14	0 04

2. Brain from an 8 months old foetus, taken 3.5 hrs after death. Diagnosis: Heart failure.

μg g

	Noradrenaline	Dopamine
Caudate nucleus	0.07	1.27
Putamen	0 08	2.11
Globus pallidus	0.07	0.31
Hypothalamus	0 24	0.00
Corpora quadrigemina	0.14	0.12
Basis pedunculi	0.00	0.15
The midbrain except the corpora quadrigemina and the basis pedunculi	0.20	0 21
Floor of the 4th ventricle	0.28	0 06

In the medulla oblongata some noradrenaline was found in the part containing the roof of the 4th ventricle. In this part the nuclei of several cranial nerves and another part of the reticular formation are found. No dopamine could be detected here. In the cerebellum no significant amounts of catechol amines could be detected. The localization of the two catechol amines in brain is schematically shown in Fig 2.

Two foetal brains have been examined, and the results are given in Table III The first of them was taken from a 5-month-old foetus. In this case the basal ganglia, the thalamus, the hypothalamus and some adjacent structures were analyzed together Small amounts of noradrenaline could be detected in this sample but the dopamine level was proportionally still lower The other brain examined was taken from an 8-month-old foetus, where a more detailed dissection could be performed. In this case some noradrenaline was found in the hypothalamus, the midbrain and the floor of the 4th ventricle Dopamine in a concentration of about 1 and 2 μg was found in the caudate nucleus and the

Table II. Distribution of 5-hydroxytryptamine in the human brain

μg/g

	Age of the patient 52 / Hrs after death 2 / Diagnosis: Heart failure	78 / 2 / Myocardial infarction	73 / 1.5 / Mammary cancer	43 / 2 / Diabetes	Mean
Cortex					
Frontal lobe		0.04			0.04
Occipital lobe		0.06			0.06
Hippocampus + olfactory bulb			0.11		0.11
Septum pellucidum		0.03			0.03
Basal ganglia					
Caudate nucleus	0.26	0.22	0.37	0.24	0.27
Putamen	0.22	0.23			0.23
Globus pallidus	0.08				0.08
Thalamus					
Medial nuclei	0.13		0.35		0.24
Lateral nuclei	0.13		0.30		0.22
Hypothalamus					
Anterior part			0.31	0.38	0.33
Intermediat part			0.32	0.64	0.48
Posterior part			0.29	0.30	0.30
Pons		0.19			0.19
Medulla oblongata					
Dorsal part containing the floor of 4th ventricle			0.50		0.50
Ventral rest			0.31		0.31

putamen respectively. Small but significant amounts were recorded in the globus pallidus and structures of the mesencephalon.

Some analyses on 5-hydroxytryptamine have been performed on samples from adult human brains (Table IV). This amine seemed to be more evenly distributed than the two catechol amines, concentrations between 0.2—0.5 μg per g being found in most of the tissues examined.

Discussion

Noradrenaline in the human brain is localized in a similar way as in the brains of other mammalian species, which have been investigated earlier (VOGT 1954 BERTLER and ROSENGREN 1959 b) Thus the highest concentrations of this amine were found in the parts which generally are considered to contain the central representation of the sympathetic activity This includes both the central representation of the peripheral adrenergic nerves and that of the adrenal medulla. A neurogenically induced release of catechol amines from the adrenal medulla is often used as a criterion of activation of sympathetic centers. It is known, however that the activity of the centre of peripheral adrenergic nerves and that of the adrenal medulla may vary independently of each other Insulin hypoglycemia, for example, activates the adrenal medulla via its centers in the brain without an appreciable activation of the adrenergic nervous system. On the contrary during muscular work the adrenergic nervous system is activated without much engagement of the drenal medulla (V ENDSALU 1960) The results of this in estigation indicate that the hypothalamic nor adrenaline is present t higher concentration in the anterior and intermediate parts. It is from this fact, however impossible to conclude that these parts of the hypothalamus are the site of sympathetic centres" nd one can put forward the question, if noradrenaline always should be found at centers with "sympathe tic activities" It may as well be a transmitter in quite a different system t a high synaptic level, just as acetylcholine is the transmitter between the pre- and postganglionic fibers of the adrenergic nervous system.

Dopamine, on the other hand, is strongly localized to structures which are involved in the motor functions of the organism, i. e. the basal ganglia and structures in close relation to these nuclei, such as the substantia nigra. The distribution w thin the basal ganglia is interesting too, as most of this amine was found n the phylogenetically youngest parts of this brain structure.

In a recent investigation SANO et al. (1960) has found larger amounts of dopamine in the caudate nucleus and the putamen than we have observed in this investigation. They also reported the occurrence of considerabl amounts of the amine in structures with no or very little connection to the basal ganglia. This discrepancy is probably due to methodological differences. As the conden sation reaction with ethylene diamine is not specific for catechol amines t is possible that some other material has been estimated, too.

From the data now available it may be concluded that noradrenaline in the brain can be of importance for higher centres of the adrenergic nervous system or of the adrenal medulla or both but t may be involved in other cerebral functions as well.

Dopamine is concentrated to structures about the function of which ery little is known, except that they in some way are nvolved in the motor functions of the organism. Results from surgical and pathological studies indicat that

the activity of the globus pallidus is i. a. influenced by the striatum and the substantia nigra. The globus pallidus is said to be the older and dynamic central nucleus in the basal ganglia. When pathological changes occur in these regions it may result in involuntary movements or muscular rigidity (see MARTIN 1959). Dopamine may play a role for the function of these nuclei and this assumption is supported by the fact that reserpine, which depletes the brain of dopamine (CARLSSON et al. 1958) can produce side effects resembling those seen in patients with lesions in the basal ganglia or the substantia nigra. Further reserpine has in some cases a favourable effect in patients suffering from Huntington's chorea (GRUMO and SUNDBY 1947, BRANDRUP 1960) where the pathological changes specially are localized to the putamen. In cases of Morbus Parkinson and postencephalitic Parkinsonism, a significant decrease of the dopamine content in the neostriatum may be seen (EHRINGER and HORNYKIEWICZ 1960).

Catechol amines in foetal brains

The preliminary results from foetal brains indicate, that noradrenaline and dopamine were present in a brain from a 5-months-old foetus. The concentration of dopamine was however very low. In a three months older foetus the dopamine concentration had nearly reached the levels found in the adult human brain. The noradrenaline concentration seemed to be increased, too, but the figures indicate, that dopamine had increased at a higher rate. It is possible, that this amine not only is phylogenetically but also ontogenetically a young substance in the brain.

Occurrence of 5-hydroxytryptamine

Results from the few investigations made on 5-HT in the human brain showed low figures, possibly due to a very rapid break down of the amine. The distribution, however, seemed to be similar to that found in dog brain by BOGDANSKI, WEISSBACH and UDENFRIEND (1957) with the highest concentrations in the hypothalamus, but appreciable amounts also being present in the medulla oblongata, the striatum and the thalamus (Table IV). The figure obtained by these authors from the hypothalamus of a human brain is of about the same order of magnitude as that presented in this investigation. The thalamus is the only structure examined with relatively high 5-HT-concentration and very low catechol amine level.

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Acta physiol. scand. 1961 51 108—115

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Brief Cyclic Variations in Some Sexual Functions of the Male Rabbit

By

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Abstract

G Degerman and J E. Kihlström. *Brief cyclic variations in some sexual functions of the male rabbit.* Acta physiol scand. 1961 *51* 108—115. — It has been demonstrated by means of two different statistical methods that at least two sexual functions of male rabbits, the volumes of daily ejaculates and the intensity of the sexual drive are subjected to cyclic variations. The length of the cycle varies individually from 5 to 6 days, and is in some individuals superimposed by a longer period.

In contrast to the well-known and thoroughly studied estrus cycle of female animals, related phenomena are practically unknown in males. There exist only a few reports indicating the occurrence of sexual periodicity in males, one dealing with the sperm count (Doggett 1956) another with the volumes of the ejaculates (Kihlström 1958) These observations are of interest from a theoretical as well as from a practical point of view and justify a more systematical investigation, including a study of possibly existing periodic variations in sexual processes not connected with the genital secretions. In addition to the volumes of the ejaculates we therefore have chosen to study the sexual drive of the animals.

Material and methods

From Oct. 8, 1959 to Jan. 22, 1960 semen was daily collected from seven male rabbits (Swedish White Landrace) ranging in age from 10 to 31 months. The environment of the animals was maintained as constant as possible They were thus given the same sort of food once a day at the same hour Except for a few days at the beginning of the experiments the bucks were kept in separate cages which were not moved. The collecting of semen was performed at 1 p. m., and the animals always yielded their ejaculates in the same sequence, thus avoiding varying visual stimulation.

One ejaculate per day was collected from each rabbit by means of an artificial vagina, and the semen transferred into small test tubes. The volumes of these tubes up to a mark in the glass wall were known with great accuracy. By adding distilled water or in some cases physiological salt solution, from a burette up to this mark the volume obtained in the tube could be determined with a high degree of precision. However, a part of the ejaculate may remain in the vagina, thus decreasing the exactness of the determinations. The error was therefore estimated by measuring the volumes recovered in the test tube after having placed precisely known volumes of semen in the vagina. This gave a standard deviation of 0.05 between paired determinations.

Six per cent of the ejaculates contained a gelatinous coagulum which was removed before the determination of the volume. About three per cent of the ejaculates were contaminated with urine which is easily observed by its colour and odour. Such ejaculates, restricted mainly to two individuals, have been excluded.

Sometimes the animals showed the ejaculatory behaviour without delivering any seminal fluid. This being the case the volumes were noted as zero. Each day the artificial vagina was presented to the animal during a maximum time of 5 min. If no ejaculate was obtained within this time the vagina was withdrawn, and the buck looked upon as refusing to copulate. In all about 700 ejaculates have been studied, the mean volume being 0.38 ml.

In order to estimate the intensity of the sexual drive the time elapsing from the moment, when the vagina was presented to the animal up to the moment, when the animal had mounted the skin and begun the pelvic thrusts was measured by means of a stop-watch. This space of time varied widely, the average being 16.9 sec.

We have looked upon the pelvic thrusts as being a better terminal point of the time measured than the ejaculation (cf. WALTON 1949). Of course these two phenomena are both dependent upon exogenous as well as endogenous factors. The moment of ejaculation is thus intimately dependent upon the skilfulness of the person operating the vagina, and is evoked when the penis comes into contact with the warm rubber tube of the vagina. The beginning of the pelvic thrusts on the other hand is independent of the position of the vagina. Nevertheless many exogenous factors may influence the time measured. However, it is very improbable that these exogenous factors should vary rhythmically. Besides it seems likely that the sexual drive is the most important factor in evoking the pelvic thrusts. Consequently we may look upon this time, t, as being approximately inversely proportional to the libido of the animal. However, in diagrams as well as in statistical treatment, it would be preferable to use an expression, in which the figure mounts and falls with the intensity of the sexual drive. In order to get such a measure of libido we have used the value $10 \times t^{-1}$ expressed in an arbitrary unit, called libido unit. It must be pointed out that this measure presupposes that the vagina is presented to the animals under standard conditions. This could not be performed with rabbit no. 2 because this animal often needed the presence of a doe in order to be sexually excited. No measures of libido could consequently be obtained.

The statistical treatment of the data obtained has been performed in two different ways. Firstly we have used a test for randomness in series of numerical observations, elaborated by KERMACK and MC KENDRICK (1937). In this test figures greater than either of their immediate neighbours in the series are defined as maximum numbers. The interval between two such maximum numbers, expressed in time units, in our case days, is called a gap, both maximum numbers being included within the gap. In series of random numbers the theoretical distribution of gaps of different sizes has been calculated by KERMACK and MC KENDRICK (1937). By means of χ^2-analysis this theoretical distribution is then compared with the distribution found in the material to be tested. The method also involves a calculation of the error of the mean size of the gaps, which gives the

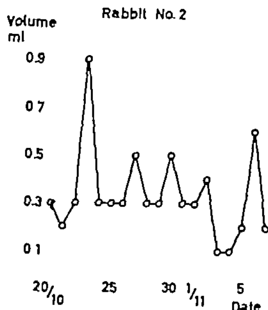


Fig. 1. Daily variations in the volumes of the ejaculates.

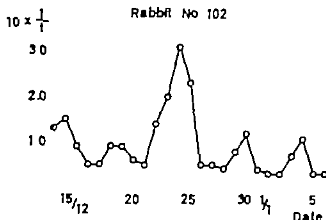
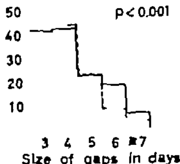


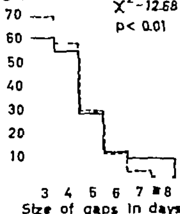
Fig. 2. Daily variations in the sexual drive. t = time in seconds from the presentation of the artificial vagina up to the beginning of the pelvic thrusts.

quotient $\frac{d}{\sigma}$ as an expression of the difference between observed and theoretical populations of gaps.

Using this method the only relevant fact is the interval between maximum numbers, the absolute magnitude of these numbers being irrelevant. In practice this means that very small and only occasionally occurring maxima are of the same importance in the calculations as greater and more pronounced maximum values. This in [illegible] gives *surplus of small gaps*.

The other method is known as periodogram analysis which includes harmonic analysis of the periods obtained (cf. [illegible] 1917). In contrast to the method of Kendall and Kermack 1937 the periodogram analysis is not influenced by small and only occasionally occurring peaks. Besides it permits us to calculate the length of the true period and to decide whether only one period exists or whether more than one period superimposed upon each other





— Observed

Theoretical

Fig. 3. The observed distribution of the sizes of the gaps between maximum volumes of the ejaculates, compared with the theoretical distribution to be found in series of random numbers.

— Observed

--- Theoretical

Fig. 4. The observed distribution of the sizes of the gaps between maximum measures of libido compared with the theoretical distribution to be found in series of random numbers.

It is also possible to estimate the probability that the cyclic variations found are statistically different from random variations. The periodogram analysis has been employed for studying possibly existing cyclic variations up to length of 14 days.

Table I. Test for randomness. Comparisons between distributions of gaps

	χ^2		P	$\overline{\sigma^2}$	P
Volumes of the ejaculates	16.50	4	< 0.001	10.40	< 0.005
Measure of libido	12.68	4	< 0.01	18.57	< 0.0003

Table II. Mean characteristics of the periods found

	Volumes of the ejaculates	Libido
Gap between maximum numbers (both maxima included)	4.31 days	4.36 days
Run down (maximum and minimum included)	2.61 days	2.60 days
Run up (minimum and maximum included)	2.70 days	2.74 day
Mean difference between maximum and minimum numbers	0.4 ml	1.3 libido unit

Table III Periodogram analysis

= the amplitudes of the harmonic periods k = the quotient between the square of amplitude examined and the mean square of the check amplitudes p = the probability that the results are *not* due t real periodic cause. Significant values in italics.

A. Volumes

Rabbit No.	Trial periods in days: 3	4	5	6	7	8	9	10	True periods in days
2	7.7 k = 6.0 *p 0.0025*	12.0 k 14.8 *p < 8 10^{-7}*	6.0 k 3.7 *p < 0.05*					= 4.4 k = 2.0 p = 0.14	3.7
28	22.16 k 9.1 *p < 3.4 10^{-}*			= 14.0 k = 3.6 *p < 0.05*					3.4 6.4
39	1.4 k 0.13 p < 0.9			= 10.6 k = 7.5 *p < 0.0025*					5.8
44	8.5 k 3.8 *p < 0.05*	8.3 k = 3.6 *p < 0.05*	= 8.2 k 3.4 *p < 0.05*			= 8.7 k = 3.9 *p < 0.05*			3.5 8.5
47	12.1 k 5.0 *p 0.007*		= 11.2 k = 4.3 *p < 0.018*						3.4 5.5
102	= 8.1 k 8.1 *p < 3.4 10*	17.3 k = 36.6 *p < 9.4 10^{-}*	= 11.4 k = 10.2 *p < 1.1 10^{-7}*						4.4
103	a = 15 k = 7.5 *p < 0.0025*		= 6.8 k = 1.6 p < 0.22						3.3

D. Libido

Rabbit No	Trial periods in days: 3	4	5	6	7	8	9	10	True periods in day
1	No figures obtained								
28	= 39.1 k = 7.6 $p < 0.0025$	42.5 k 9.0 $p < 3.4$ 10^-		= 36.5 k = 6.6 $p < 0.0025$					3.5 6.5
30	= 36 k 2.0 p 0.14	61 k = 5.8 $p < 0.007$			= 36.5 k = 2.1 $p < 0.14$				3.4
41	7.8 k 0.6 $p < 0.5$		= 20.6 k = 4.5 $p < 0.01$						5.3
47	45 k = 6.2 $p < 0.0025$	= 57 k = 10.0 $p < 4.5$ 10^-						= 53 k = 6.7 $p < 3.4 \times 10^{-1}$	3.6 10.3
102	41 k 6.8 $p < 0.005$	= 9.9 k = 0.1 $p < 0.67$							3.2
103	7.2 k = 0.2 p 0.82	= 12.2 k 0.5 $p < 0.67$	44.5 k = 7.1 $k < 0.0025$	= 21.7 k = 2.2 $p < 0.14$					5.3

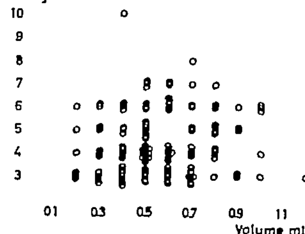


Fig. 5. The sizes of the gaps between maximum volumes plotted against the first maximum volume within the gap in question.

Results

The values obtained have been plotted in diagrams, as in Fig. 1 and 2. When using the test for randomness, devised by KERMACK and MC KENDRICK (1937) it was necessary to pool the values from all rabbits in order to get a sufficiently large statistical material. The comparisons by means of χ^2 analysis between the distributions of gaps observed and of those calculated are given in Fig. 3 and 4. As seen from these diagrams we must look upon the series of numbers obtained as not being random. In other words, we have strong indications of a really existing periodicity in the volumes of the ejaculates as well as in the intensity of the sexual drive. The same results are obtained by calculating the quotient $\frac{s}{\sigma^2}$ (See Table I).

The mean characteristics of the period found by this method are given in Table II. As seen from the table the two properties studied show mean cycles nearly identical in length and form.

When using the periodogram analysis figures obtained from different individuals could be treated separately. The results are summarized in Table III. Beginning with the volumes of the ejaculates this table reveals that all animals show a primary cycle with a statistically probable length varying from 3.3 to 5.8 days. Besides, in 3 out of 7 rabbits we find a secondary cycle superimposed upon the shorter one. This longer cycle varies in length from 5.5 to 8.5 days.

As mentioned earlier it was impossible to estimate the libido of rabbit no. 2. The remaining six rabbits all show periodic variations with a primary cycle varying individually in length from 3.2 to 5.5 days (See Table III). In two

individuals this shorter period is superimposed by a longer one, the length of which is 6.3 and 10.3 days, respectively

In one animal rabbit no. 28, we find the two properties studied to have approximately equal probable lengths of the cycles. Nevertheless, the cycles are not synchronous.

Discussion

As to the causes of the periodicities found it is possible that an unusually big ejaculate may be followed by a strikingly long interval before the sexual glands have produced enough secretions to yield another big ejaculate. If this is the case we should expect to find a positive correlation between the maximum volumes and the intervals following up to the next maximum volume. However no such correlation could be revealed (See Fig 5)

The periodical variations may also depend upon a varying irritability of the nerves supplying the genital organs, or the genital organs themselves may vary in their response to the sexual hormones. However the parallelism in length between the periodical variations found by the present authors and those demonstrated by DOGGETT (1956) strengthens the impression that these variations are different expressions of cyclic variations in the hormonal status, most likely in the circulating amount of androgen hormones. Work is in progress with a view to elucidate these problems.

In most handbooks the length of the estrus cycle of female rabbits is said to be 7 to 10 days (ASDELL 1946, PARKES 1956) However HAMILTON (1951) has found a female cycle of 4—6 days, which is in agreement with the length of the male sexual cycle found in the present work.

We are indebted to the Head of the Institute, Professor P E. LINDAHL, for valuable discussions. Financial support from Lars Hierta Memorial Foundation is gratefully acknowledged

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Acta physiol. scand. 1961 51 116—135

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Hemodynamic Studies with Differential Pressure Technique

By

I. G. Porjé and B. Rudewald

Received 13 September 1960

Abstract

Porjé, I. G. and B. Rudewald. *Hemodynamic studies with differential pressure technique.* Acta physiol. scand. 1961. *51.* 116—135. — A theoretical analysis of the fluid motion in circular elastic tube is presented. The formulae for computation of pulsatile flow and other hydrodynamic data are discussed. Apparatus and technique for recording differential pressure are described. Results of studies of the hydrodynamics in model circulation system show that the theory is valid. For kinematic viscosities ranging from 1—8 cst the differential pressure is in the main unaltered. Original differential pressure records from the ascending aorta in cases with normal aortic valves are presented and their features described. Blood velocity curves computed from such curves are presented. To illustrate the use of the method the effect of the Valsalva manoeuvre and a nitrite (Etrynit, Bofors) upon the aortic blood motion are demonstrated.

Heart catheterization has opened possibilities for quantitative determination of the cardiac output per minute and the stroke volume according to the Fick or the Hamilton principles. Thereby valuable clinical and physiological data about the circulation in intact man is obtained. These methods give mean values for the flow during a certain interval of time but no information about the dynamics of a particular heart stroke. For the direct measurement of the flow there are several types of flowmeters but as yet none can be used safely to measure the instantaneous aortic blood flow in intact man. Our work has been devoted to the development of a method for the study of the dynamics of the aortic blood motion, based upon differential-pressure curves recorded from the aorta.

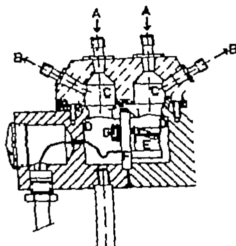


Fig. 1. Diagram of the pressure transducer. For description, see text.

The theoretical and experimental problems met with in the hemodynamics of the arterial circulation are extremely intricate. These problems have recently been reviewed by McDonald and Taylor (1959). The theoretical works by Womersley have particularly contributed to the understanding of the dynamics of the pulsatile flow. In earlier works (1957 a, b) we have reported on the dynamics in the ascending aorta in human beings. Fry *et al.* (1956) reported on studies of the blood flow in the dog's aorta with a technique similar to ours. This group has continued their work and also investigated the aortic blood flow in human beings.

The motion of a Newtonian fluid in an elastic tube can be described by partial differential equations. In their general form these equations cannot yet be solved in an explicit form. By suitable hypotheses, however, about the deformations of the tube and the effect of the viscosity we can solve the equations. Our aim is to achieve formulae relating flow or velocity to some physical magnitudes that can be measured in vivo. It is obvious that the non-Newtonian behaviour of the blood, the visco-elastic properties of the vessels and their biological activity will be factors that limit the theoretical approach.

For the theoretical part of this study the reader is referred to the addendum.

Apparatus

As the reliability of the pressure difference curve is very important for our calculations, the demands on the recording system must be high. Our first Δp-curves from human beings were obtained with two separate catheters attached to a particular differential pressure transducer. As accelerations and bendings of the catheters may cause distortion of the Δp-curve, double lumina catheters should be preferred. As no suitable catheters were available for our purpose, different types were designed in cooperation with

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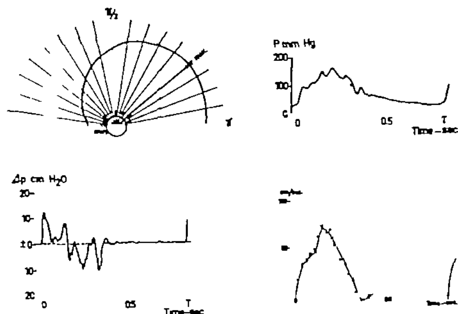


Fig 3 Upper left diagram shows the eccentric that is driven by motor t constant p m. When the eccentric turns from to liquid is ejected from the pump. During the rest of the revolution there is zero flow Redrawn differential pressure curv (1 cm H_2O) is shown below Computed values for fluid velocity (in cm/sec.) are indicated by heavy dots ( ) Corresponding values calculated from the profile of the eccentric are plotted for comparison (The "stroke volume is 66 cm³ Computed value 64 ± 4 cm³

Our present system of registering w th double lumina catheter length 100 cm, nd outer diameter corresponding t USCI no. 6,5 has the frequency response shown in Fig . There is flat amplitude response for frequencies lower than about 14 hz and the phase-lag is negligible for the same range of frequencies. It is observed that for higher frequencies there is resonance peak with a maximum t about 26 hz This means that signals containing frequencies within this range will be distorted I the frequency spectrum of the aortic pressure curve the amplitude of harmonics higher than the 7-th is only a very small percentage of the fundamental, (ref. no. 7) and thus must be ery low Therefore the dynamic properties of the recording system will be sufficient for the measurements referred to here It is however desirable to improv the high frequency response as w ll be done in new pparatus.

The catheter is provided with lateral openings and the tip is filled w th heav metal. The increased mass renders the catheter tip less sensitiv t small high frequency ibrations. The pressure difference has been recorded in circulation model and human beings with type of catheter A simple test of the performance of the recording system can be carried out by shifting the catheter endings to opposit sides of the pressure transducer This manipulation will giv curve that is the original one with negative sign. As even ery small air bubbles will cause asymmetry they re easily detectable by this test.

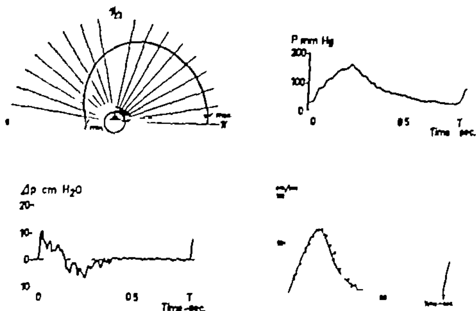


Fig. 4 Excentric with more smooth curvature and increased length of the ejection phase Differential pressure and computed ejection arranged before The stroke volume 1 66 cm³ Computed value 65 ± 4 cm³

Fig. 5. Excentric with steep ascent and increased stroke volume 80 m) Computed value 81 ± 5 cm³

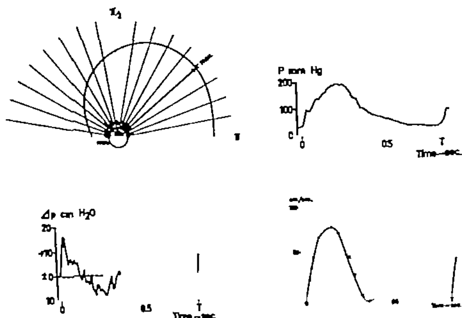


Fig. 6. The same eccentric as in fig. 8 with less steep ascent. The duration of the ejection phase about the same. The "stroke volume" is 81 cm³ Computed value 76 ± 5 cm³

Model experiments

I previous papers (PORJÉ and RUDEWALD 1955, 1957 a, b, 1959) w referred to experimental studies of the hydrodynamics in circulation model.

The main features of the model circulation system have been described earlier A brief description of the experimental conditions and some technical details will be given for the sake of completeness.

The output haracteristics of the pump are determined by the profile of the eccentric. The "stroke olume the "systolic phase and the velocities in the ejection flow can thus be varied simply by using different profiles of the eccentrics. The p. m. of the eccentric is constant and the period T = 0.82 sec. The temperature of the circulating liquid is 18 C. An elastic rubber tube, length 25 cm, is attached to the outlet of the pump and connected to an elastic plastic tube with plexiglass muff. The volume elasticity modulus of the rubber tube is determined experimentally to be 930 mm Hg The double-lumen catheter is inserted in the elastic plastic tube through branch in the distal part of the tube and positioned with the foremost opening just outside the outlet of the pump The differential pressure transducer and the catheter is filled with boiled w ter and carefully aired before the insertion of the catheter Static calibration is carried out for both sides of the manometer The Δp-curve is recorded and then the catheter endings are shifted to opposite sides of the pressure transducer to test the symmetry Only when the integrated bsolute values of Δp and — Δp are nearly identical, is he difference pressure curve accepted as correct. Disturbances due to small vibrations may cause minor differences in the shape of the curves but as rule it is possible to decide by inspection whether the Δp-curve is correct or not.

Series of Δp-curves are recorded with different profiles of the eccentric. The circulating liquid is water In Fig. 3—6 the profile curve of the eccentric, the corresponding Δp-curves and computed velocities are demonstrated. From the graph of the eccentric the velocity of the plunger can be calculated for short intervals of time (0 022 sec) nd alues for the outflow velocity are plotted for comparison with values computed from formula (17) It is observed that the computed and actual values compare well.

The effect of the viscosity is studied by dding a viscosity increasing substance (methocel, ASTRA) to the water circulating in the model. The Δp-curves for kinematic viscosities ranging from 1—9 centistoke ha nearly the same shape The differential pressure tends to be slightly positive as the viscosity increases, but values for this positivity are not precise enough to permit any quantitative estimation.

Our experimental observations from this particular model circulation system may be summarized thus

1 Computed values for the instanteneous velocity and the stroke olume are in good agreement with given values.

2. When the outflow pattern from the pump is slightly varied the shape of the Δp-curve is changed in a distinct way

3 The shape of the Δp-curve is in the main unaltered when the viscosity of the circulating liquid ranges between 1—9 cst and the deviation of the mean differential pressure from zero is not significant.

In these models experiments w find that fluid velocity and stroke volume can be computed with sufficient accuracy from formula (16) and (17) The pulse-w velocity of the tube is determined from static measurement of the volume elasticity modulus according to Hallock and Benson. From their measurement on utopsy material w find that this tube would correspond to a rather rigid aorta.

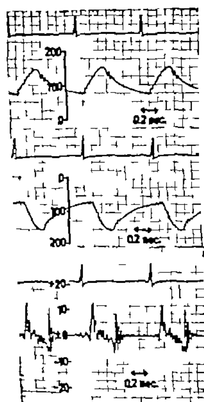
For kinematic viscosities ranging from 1—9 cst the mean Δp does not deviate significantly from zero. The viscosity is measured in an Ostwald viscometer at 18 C. At high rates of shear the blood and the fluid used here certainly behave differently

According to the Poiseuille equation the pressure drop would be at most 0.1 cm H_2O in these experiments. An accurat determination of the mean Δp of this order of magnitude is not possible with the present pparatus. From the theoretical considerations referred to, the pressure drop due to the viscosity should be negligible for short distance along the tube Experimentally w arriv at the same conclusion.

Aortic differential pressure in human beings

In 1956 I G PORJÉ and B. RUDEWALD started to record the differential pressure in the human aorta and these records presented in 1957 were as far as we know the first direct records from human beings.

At that time we used two catheters which were inserted in the aorta after bilateral puncture of the femoral artery Now the special double-lumen catheter is used for recording the aortic differential pressure The catheter is inserted percutaneously in the femoral artery The vessel is punctured under local anesthesia w th a modified Gidlund cannula The catheter is advanced to the ascending aorta under fluoroscopic control The catheter is curved in its foremost part to facilitate its passage through the aortic arch (ÖDMAN and PHILIPSON 1958) Two perpendicular radiograms are exposed to check the position. From the radiograms the distance (L) between the catheter openings can be calculated. The catheter is perfused with heparinized saline



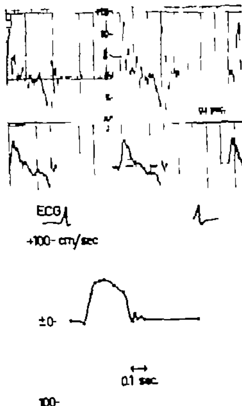


Fig. 7 a. Fig. 7 b.

Fig. 7 a. Ascending aortic pressure (A and B) and differential pressure ( )   38-year-old woman with normal aortic valves. ECG above the curves.

Fig. 7 b. The differential pressure with greater amplitude and the time scale streched out. Lower curves is obtained by filtering off the higher frequencies. The maximum Δp value is about 12 cm H_2O. L = 8.5 cm. Values for the fluid velocity obtained from formula (17) are plotted in bottom diagram.

during the whole investigation. Records of the pressure and the differential pressure are only taken during short intervals of time. With these precautions there is no risk for coagulation at the catheter openings. Extreme care must be devoted to the airing of the system. As mentioned previously the symmetry of the system can be checked by shifting the end of the catheter to opposite sides of the differential pressure transducer. By suitable adjustment of the stop-cocks this test can be performed quite easily. With this technique the aortic differential pressure has been recorded in cases with normal aortic valves and with aortic valvular disease, particularly in aortic valvular stenosis. The total number of cases investigated with the differential pressure technique is 31. As the recording apparatus has been subjected to modifications during the

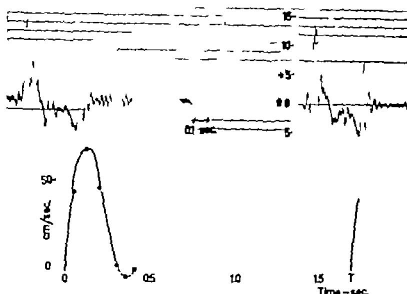


Fig. 8. Ascending aortic differential pressure (in cm H_2O) from a 64-year-old man with a total heart block. No signs of any aortic valvular disease, max. Δp is about 15 cm H_2O. L = 8 cm. Some values for the fluid velocity are plotted below ( ).

course of the investigations the material cannot be treated statistically. The cases presented here are representative for the results obtained with our present technique.

Results

In cases with normal aortic valves the Δp-curve from the ascending aorta has a characteristic pattern as shown in Fig. 7 a, b. At the onset of systole there is a distinct positive steep rise corresponding to the acceleration of the blood (the net force is directed *from* the heart). Then the Δp becomes negative and at the end of systole there is a negative dip followed by high frequency oscillations of very short duration. For negative Δp the blood is decelerated (the net force is directed *towards* the heart). During diastole the Δp is equilibrated to a smooth zero niveau. Fig. 8 shows another case with a total heart block. There were no clinical signs of any aortic valvular disease.

From formula (17) values for the blood velocity are obtained which we may first look upon as hypothetical. The velocity is assumed to be zero at the beginning of systole. There is a rapid increase of the velocity (Fig. 7 b, 8 b) to its maximum values of about 60—90 cm/sec. Then the velocity decreases to zero and this zero point agrees very well with the expected end of the ejection phase and the end of systole obtained from the PCG. During the remainder of the period (diastole) the velocity is nearly zero. In some cases small negative values are found just after the zero point. The stroke volume can be calculated if the cross-sectional area of the aorta is known e. g. from

thoracic aortography Plausible values can be obtained from statistical tables (PORJÉ 1946) With such values we find that the stroke volume would be about 60—80 ml in the cases demonstrated here. The mean velocity is found to be about 12 cm/sec.

By recording the ascending aortic pressure simultaneously with the differential pressure, the heart power and the heart work can be calculated (I. G PORJÉ and B. RUDEWALD 1957 formula 14) The distance L is measured from the radiograms to be about 8 cm in the cases demonstrated here. Furthermore we find that the mean value of the Δp is about zero — the deviation is not significant.

Discussion

With a distance of 8—10 cm between the openings in the catheter we get reliable differential pressure records and this distance also fits the length of the ascending aorta. As the foremost part of the catheter is curved this figure is reduced and it must be measured in each individual case in the radiograms. To compute hemodynamic data about the blood motion from our formula (7) and (13) the cross-sectional area of the aorta and its variation with time and the length coordinate must be known. Recent investigations by PETERSEN *et al* on the dog's aorta with simultaneous recording of the cross-sectional area and the intra-vascular pressure indicate that the dynamic behaviour of the aorta is that of a rather rigid tube. It is reasonable to assume that the human aorta should behave in much the same way This view is also supported by observations from angiographic studies of the ascending aorta made by ARVIDSSON (1960) He finds that the area change in cases with intact valves is at most 5 for a normal range of pulse amplitudes. This indicates that the simplified formulae (16) and (17) could be used with sufficient accuracy to compute instantaneous mean flow and aortic blood velocity in such cases. The instantaneous blood flow at the root of the aorta should differ only slightly from the instantaneous mean flow If sufficient experimental data are available formulae (7) and (13) can be used for more exact calculations of these data also in cases, where the cross-sectional area varies within wider limits.

The high frequency oscillation superimposed on the Δp-curve are mainly caused by rapid catheter vibrations. A slight change of the position of the catheter will alter the contents of these oscillations. When the Δp-curves from different positions are integrated for suitable intervals of time values are obtained that differ slightly The high frequency response of the recording system should however be adapted to minimize these artefacts.

The maximim differential pressure is about 13 cm H_2O in the cases presented here Then the maximum acceleration of the fluid would be about 1,600 cm sec The volume of the left ventricl has been calculated from rapid serial angiocardiography by ARVIDSSON 1960) In cases with intact mitral and aortic valves the maximum acceleration of the blood during early systole

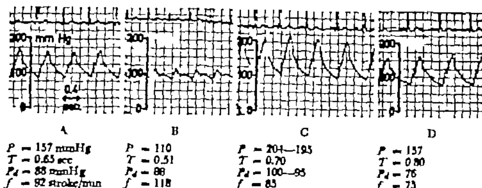


Fig. 9. Aortic pressure just above the valves during the Valsalva manoeuvre. Record A taken immediately before the strain and record B during expiration (intraoral pressure about 50 mmHg) after maximum inspiration. Record C is taken 5 sec. and recorded D 15 sec after the release of the strain. The maximum systolic pressure is denoted by P and the end-diastolic pressure by P_d. T is the period and f the heart frequency.

is calculated to be of the same order of magnitude as that obtained from the differential pressure record.

Of particular interest is the observation that the mean Δp does not differ significantly from zero. It seems justifiable to conclude that the viscosity forces are small compared to the inertial forces in the ascending aorta.

As mentioned above the differential pressure technique is particularly suitable for the study of rapid alterations of the dynamics of the arterial blood flow. To illustrate this we shall present some preliminary studies of the circulatory effect of the Valsalva manoeuvre and a rapidly acting drug. The use of the method for diagnostic purposes in aortic valvular stenosis has been dealt with earlier (PORJÉ and RUDEWALD 1959).

The differential pressure during the Valsalva Manoeuvre

The Δp-curves presented so far are obtained during a steady state. In order *to study the Δp-pattern when the left ventricular output is changing rapidly* the patients are instructed to carry out the Valsalva manoeuvre. The pressure just above the valves is recorded during the manoeuvre (Fig. 9). Some Δp-curves from a sequence of records are shown in Fig. 10 together with computed velocity curves. We find that the altering of the shape of the Δp-curve is characterized by a slightly increased amplitude at the beginning of the manoeuvre, followed by a strong decrease. When the patient starts to breathe regularly the Δp is for some strokes of slightly increased amplitude but soon it returns to the basic amplitude. The relative change of the corresponding stroke volumes are given in Fig. 10.

When a small dose of a nitrite 5—10 mg, (Erynit, Bofors) is given to patients a transient decrease of the central blood pressure is observed in some cases. The aortic differential pressure and computed blood velocity for such a case

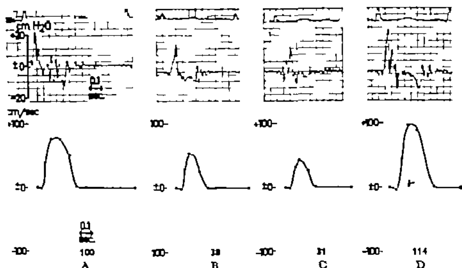


Fig 10. Ascending aortic differential pressure during the Valsalva manoeuvre. A before, B and C during strain and D 4 sec. after the release of the strain. Computed values for the blood velocity are shown below. The stroke volume before the strain is arbitrarily put to 100.

ECG
200- mm Hg
100-
0-
0.4

P = 135 mmHg T = 0.89 sec.
P_d = 71 mmHg
f = 67 stroke min

P = 124 T = 0.71
P_d = 70 f = 84

P = 90 T = 0.84
P_d = 56 f = 71

P 86 T 0.89
P = 48 f = 67

P = 84 T = 0.89
P_d = 30 f = 67

P = 133 T = 0.67
P_d = 70 f 90

Fig 11 a.

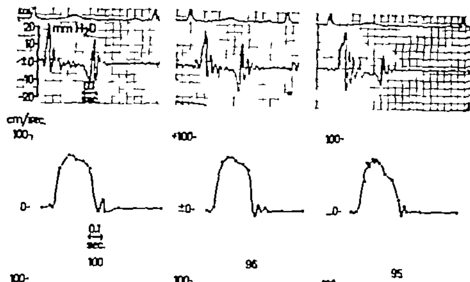


Fig. 11 b.

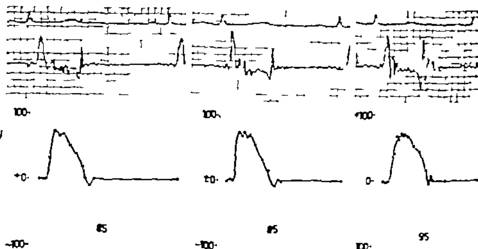


Fig. 11 c.

Fig. 11 a—c. The effect of a small dose of ... Ervrun, Bofors, upon the ascending aorta pressure and the differential pressure ... 48-year-old woman with pseudo-xanthoma elasticum. No clinical signs of any aortic valvular disease. P systolic pressure, P_d diastolic pressure, T the period and f the heart frequency. Differential pressure and computed blood velocity are shown in fig. 11 b and c. The stroke volumes are given in percent of the stroke volume before ervrun is administrated. The records are taken with 5 min intervals.

is shown in Fig. 11. It is observed that the stroke volume is reduced concomitantly with the decrease in the central blood pressure. Pharmacological studies of this kind are going on.

This study was aided by grants from the Knut and Alice Wallenberg Foundation and the Swedish Medical Research Council.

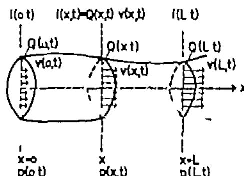


Fig. 12. Sketch of an elastic tube illustrating the symbols used. See text.

Addendum

In the theoretical treatment the ascending aorta is represented by a uniform non branching elastic circular tube. For our purpose it is convenient to discuss two alternatives separately: A. The motion of non-viscous fluid in an elastic, circular tube and B. The motion of viscous fluid in rigid circular tube. The following symbols are used.

t time
the length coordinate taken along the axis of the tube
L the length of the tube
R the radius of the tube
$Q(x,t)$ the cross sectional area of the tube
Q_o the cross sectional area at certain pressure *e. g.* the end-diastolic pressure
$\varepsilon(x,t)$ the relative change of Q defined by $Q(x,t) = Q_o\,[1 + \varepsilon(x,t)]$
$p(x,t)$ the lateral pressure
$v(x,t)$ the xial fluid velocity
(x,t) the rate of flow $I(\ ,t) = Q(x,t)\ v(x,t)$
ϱ the density of the fluid
μ the iscosity of the fluid
the kinematic viscosity
$\left(= \frac{\mu}{\varrho} \right)$
the angular frequency

The motion of non-viscous fluid in an elastic circular tube

I. W apply the momentum equation to the fluid motion in an elastic tube of leng h l. The relation between the rate of change of the momentum and the forces acting up n he fluids is given by the following vectorial equation (*e. g.* Müller 1932)

$$(1)\quad \frac{\partial}{\partial t}\int_S \varrho \vec{\ } \, ds + \int_F \varrho \quad df \ \vec{\ } = -\vec{P}$$

The integration should be carried out over the space S containing the fluid and it boundary surfaces F (Fig. 12

The -component of eq (1 is

$$(2)\quad \frac{d}{dt}\int^L \varrho\ Q \quad dx + \varrho\left[Q(L,t)\ v^2(L,t) - Q(\ ,t)\ \ v^2(o,t)\right] = Q(o,t)\ p(o,t) - Q(L,t)\ \ p(L,t)$$

For oscillatory flow L should be small compared to the wavelength. Then the axial component of forces from the tube walls can be neglected. With $i(x,t) = Q(x,t) \cdot v(x,t)$ eq (2) can be written

$$(3) \quad L\frac{di_m(t)}{dt} = \frac{1}{\varrho}\Big[Q(0,t) \cdot p(0,t) - Q(L,t) \cdot p(L,t)\Big] + \frac{i^2(0,t)}{Q(0,t)} - \frac{i^2(L,t)}{Q(L,t)}$$

where $i_m(t)$ is the mean flow within the tube $i_m(t) = \frac{1}{L}\int_0^L Q(x,t) \cdot v(x,t)dx$

The quotient between the cross sectional areas at $x = 0$ and $x = L$ is

$$\frac{Q(L,t)}{Q(0,t)} = \frac{1+\varepsilon(L,t)}{1+\varepsilon(0,t)} \approx 1 - \Delta\varepsilon \quad \text{where} \quad \Delta\varepsilon = \varepsilon(0,t) - \varepsilon(L,t)$$

As $\varepsilon(x,t)$ is positive and small compared to 1 then $\Delta\varepsilon << 1$ and terms multiplied by $\Delta\varepsilon$ can be neglected.

With $\Delta p = p(0,t) - p(L,t)$ and $\Delta i = i(0,t) - i(L,t)$ eq (3) becomes

$$(4) \quad \frac{di_m}{dt} = \frac{1}{\varrho L} \cdot Q(0,t) \cdot \Delta p + \frac{\Delta i[i(0,t) + i(L,t)]}{L \cdot Q(0,t)}$$

The equation of continuity for the tube is

$$(5) \quad \frac{d}{dt}\int_0^L Q(x,t)dx = \Delta i$$

The mean value of Q within $0 \le x \le L$ is $Q_m = \frac{1}{L}\int_0^L Q(x,t)dx$

Combining eq (4) and (5) we get after integration

$$(6) \quad i_m(t) = \frac{1}{\varrho L}\int_0^t Q(0,\tau) \cdot \Delta p(\tau)d\tau + \int_0^t\left[\frac{i(0,\tau) + i(L,\tau)}{Q(0,\tau)} \cdot \frac{dQ_m(\tau)}{d\tau}\right]d\tau + C$$

Where C is a constant to be determined from some boundary condition e.g. $i_m(0) = 0$. Then $C = 0$

Put $i_m(t) = J_F(t) + J_K(t)$ with $J_F = \frac{1}{\varrho L}\int Q(0,\tau) \cdot \Delta p(\tau)d\tau + C$

and $J_K = \int\left[\frac{i(0,\tau) + i(L,\tau)}{Q(0,\tau)} \cdot \frac{dQ_m(\tau)}{d\tau}\right]d\tau$ with $i(0,t) + i(L,t) = 2J_F$ we get

$$(7) \quad i_m(t) = J_F(t) + \int_0^t\left[\frac{2J_F(\tau)}{Q(0,\tau)} \cdot \frac{dQ_m(\tau)}{d\tau}\right]d\tau$$

Comments

Eq (7) could be used for determination of the instantaneous mean flow through an elastic circular tube. What data from the ascending aorta are necessary for such a determination and how should they be obtained? The differential pressure $\Delta p(t)$ must be recorded by some

suitable device for example by means of catheter or needle. The cross-sectional area $Q(\ ,t)$ and mean value $Q_m(t)$ must be determined by for example, thoracic aortography. Then the non-corrected flow $\mathfrak{J}_n$ and the correctional term, $\mathfrak{J}_K$ can be computed graphically.

II. Let us now suppose that the aortic pressure gradient, $\frac{\partial p}{\partial}$ the cross sectional area of the aorta, Q, and its derivative with respect to $\frac{\partial Q}{\partial}$ are measured t the root of the aorta viz. at $x = a$. Then the following equations can be used for the computation of the aortic blood flow.

Combining the motion equation

$$(8)\quad \frac{\partial v}{\partial t} + \quad \frac{\partial v}{\partial x} = -\frac{1}{\varrho}\ \frac{\partial p}{\partial x}$$ and the equation of continuity

$$(9)\quad \frac{\partial Q}{\partial t} + \frac{\partial Q}{\partial x} = 0$$ the following equation is obtained

$$(10)\quad \frac{dU(t)}{dt} - \frac{U(t)}{(Q)}_{=0}\left[\left(\frac{\partial Q}{\partial t}\right)_{=0} + U(t)\left(\frac{\partial Q}{\partial x}\right)_{=0}\right] = -\frac{1}{\varrho}\left(\frac{\partial p}{\partial}\right)_{=0}$$

here $U(t) = v(a,t)$

The functions to be measured are denoted by

$$f(t) = -\frac{1}{\varrho}\left(\frac{\partial p}{\partial x}\right)_{=0} \quad g(t) = (Q)_{=0}\ ;\ h(t) = \left(\frac{\partial Q}{\partial x}\right)_{=0}$$

The flow t = is denoted by $F(t) = (Q)_{=0}\ v(a,t) = g(t)\ U(t)$

The differential equation for $F(t)$ becomes

$$(11)\quad F'(t) - 2F\ \frac{g'}{g} - \frac{F^2\ h}{g^2} = f\ g$$

This equation is of the Riccatis type and it cannot be solved in an explicit form. For rigid tube eq. (11) is reduced to $F'(t) = f\ g$ with the solution $F = F_0(t)$

$$F_0(\ ) = \int f(\ )\ g(t)dt - k_0$$

The constant of integration k_0 can be determined from the condition that

$$\min.\ F_0(t) = 0 \quad \text{then} \quad k_0 = \min. \int f(t)\ \ g(\ )dt$$

The terms $F\ \frac{g}{g} + \frac{F^2\ h}{g^2}$

can be looked upon as correctional terms. W get good estimation of them by putting $F = F_0$.

B. J. Anderson, personal communication.

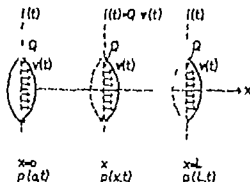


Fig. 15 See text.

Then eq (11) becomes

(12) $F = f \; g + 2F \quad \frac{g}{g} + \frac{F^2 k}{g^2}$ Integration gives

(13) $F(t) = F_o(t) + \int \left[\frac{2F_o(\tau) \; g'(\tau)}{g(\tau)} + \frac{F^2(\tau) \; k(\tau)}{g^2(\tau)} \right] d\tau - k_1$ where

$k_1 = \min \int \frac{2F_o(\tau) \; g'(\tau)}{g(\tau)} + \frac{F^2(\tau) \; k(\tau)}{g^2(\tau)} d\tau$ The stroke volume is

(14) $V = \int^{T} F(t)dt$ where T is the period

T determine the efficiency of the pump (the heart) we have to measure the pressure $p(t,t)$ at $= o$ (the root of the aorta)

The mean effect during one period is

(15) $E = \frac{1}{T} \int^{T} p(O,t) \quad F(t)dt$

Comments

If the second correctional term $\frac{F^2 k}{g^2}$ is neglected eq (13) becomes formally identical with eq (7) The functions $g(t)$ and $k(t)$ can be determined in the manner indicated abov and if the pressure gradient $\frac{dp}{dx}$ is substituted by the differential pressure over a short distance L, $\frac{\Delta p}{L}$ then eq (13 offers possibilities for more exact calculation of the flow

Discussion

For a *rigid tube* eq 7 is reduced to $i(t) = Q \quad (t) = \int \; fp(\tau)d\tau + C$

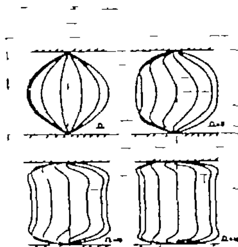


Fig. 14. Velocity profiles for the oscillatory flow of viscous, non-compressible fluid for range of values of Ω. See text. (From WOMERSLEY 1950).

is, the integration of the pressure difference will give values proportional to the instantaneous fluid velocity $v(t)$, and as Q is constant (Fig. 2) also to the instantaneous flow (t) through the tube. The distance L can be chosen arbitrarily. The shortest L, that can be obtained is determined by the limitations of the recording system.

For rather rigid tube and within restricted range of variations of the pressure amplitude the difference between in- and outflow f_1, is small quantity for short part of the tube. Then the correctional terms can be neglected and the mean instantaneous flow through the tube $i_m(t)$ can be computed from

$$(16) \quad i_m(t) = \frac{1}{\varrho L} \int Q(O\,\tau) \quad f(p\,\tau)\,d\tau - C$$

and $i_m(t)$ differs slightly from the inflow $I(o,t)$. The linear velocity $L(t)$ obtained from

$$(17) \quad L(t) = \frac{1}{\varrho L} \int f(p(\tau))\,d\tau + C$$

will approximate to the mean instantaneous fluid velocity. I differs more or less from the fluid velocity in the entrance flow o,t but the deviation should be within rather close limits. The distance L should be as short as possible.

If the tube is more elastic and/or the pressure amplitude varies within wider limits, the difference between in- and outflow f cannot be neglected even for short distance L. In this case the general equations must be used for computation of the mean flow $i_m(t)$. The flow particular point x t F can at least in principle be computed according to eq 11 if L sufficiently small.

The anatomy of the ascending aorta does not allow the pressure difference to be measured over distance greater than about 8—10 cm. The measuring points should be referred to the centre of the cross-sectional to reduce the effect of the curvature of the vessel. In practice these

conditions are difficult to ful-fil in vivo. The choice of a particular L should, therefore, first be made from experiments particularly arranged to test the validity of the theory and the performance of the recording system. W have preferred to do this in model circulation system. Experiences from studies of the dynamics of the aortic blood flow and the ascular walls will show what measuring parameters should be used.

B. The motion of viscous fluid in rigid circular tube

For stationary flow the relation between flow and pressure drop is given by the wellknown Poiseuille equation. For oscillatory flow equations ha been deduced by several research workers. We refer particularly to the excellent and extensive theoretical studies by Womersly He points out that the character of the flow is determined by non-dimensional parameter

$\alpha = R\sqrt{\frac{\omega}{\nu}}$ where R is the radius of the tube, ω is the angular frequency and ν is the kinematic

viscosity of the liquid. Velocity profiles for range of values of α have been given by for example, WELANDER (1950) (Fig 14) H uses the symbol Ω instead of α. For increasing values of Ω the velocity profil becomes more flattened in the axial part of the tube I the human aorta Ω should be bout 20. Then the fluid is mainly unsheared, except for rather thin layer close to the wall. For given tube and given kinematic iscocity the phase lag between the flow and the pressure gradient will approach 90° as the frequency increases (WOMERSLEY 1955)

FRY *et al.* (1956) has derived a formula from the Navier-Stoke differential equation for the computation of the flow in the ascending aorta from the pressure difference These assume that the frictional forces are proportional to the velocity of the fluid. Recently FRY (1959) proposes a formula with certain tentati constants. Proportional ty between frictional forces and fluid elocity is also treated by WELANDER (1950) who gives an equation for the computation of the constant for different types of flow

Comments

The application of these results to the flow near the outlet of the pump (the ascending aorta) is questionable. Even in pulsatile flow the velocity profile does not form until certain unknow distance from the tube inlet. The elocity profile may be determined mainly by the velocities in the ejection flow and urbulence may occur For an elastic t be the situation becomes even more complex and there is no general solution yet to the non-linear differential equations valid for this type of flow Therefore it seems to be justified to use the formulae for non-viscous flow to compute the blood flow in the ascending aorta in man The errors due to viscous effects should be of minor importance compared to those from other sources There is also experimental evidence that supports this view (see page 8) The pressure loss due to the visco-elastic properties of the aortic alls are another possibl source of error For an estimation of this effect the studies on the dog aorta by PETERSEN *et al* (1960) are of particular interest (see page 11)

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(THESLEFF 1960 a). However the possibility that inactivity *per se* is responsible for the spread of chemical sensitivity deserves consideration, particularly since CANNON and HAIMOVICI (1939) have reported that an increased sensitivity to acetylcholine (ACh) follows decentralization of motor neurones in the cat.

The present study to investigate whether the lack of motor nerve impulses alters the chemically sensitive area in skeletal muscle, consisted of determining the size of the ACh-sensitive areas in muscle fibres of the tenuissimus muscles of the cat. Such determinations were made before, and at varying periods after the elimination of all afferent impulses playing upon the lower motor neurones of the subserved tenuissimus muscle. The isolation of the motor neurones was accomplished by severance of the dorsal roots below a cord transection, as described by TOWER (1937 a, b).

Methods

Complete isolation of the lower motor neurones serving the tenuissimus muscles was successfully accomplished in 9 cats. It had previously been established that the tenuissimus muscle of the cat receives its motor nerve supply through the seventh lumbar and the first spinal roots. Under pentobarbitone anesthesia, the spinal cords were transected between segments L 4 and L 5 and all dorsal roots below the transection were identified and severed. Furthermore, all ventral roots below S 3 or 4 were severed. In such an isolated preparation, the ventral horn cells remain intact, yet deprived of all afferent impulses (TOWER 1937 a, b, ECCLES 1944).

Despite the severe operative procedure and its deficit, it was possible to maintain the animals in relatively good condition for periods of from one to four weeks, the weight loss being 20–30 % or less. In three cats a sham operation was performed, only the actual isolation of the cord segments was omitted. At intervals of two days the muscles of the hindlegs were tested for the presence of electrical activity by the use of conventional electromyograph with concentric needle electrodes.

In individual muscle fibers the sensitivity to ACh was determined by iontophoretic micro-application of the drug, and by intracellular recording of the resultant membrane potential change. (For details of the experimental apparatus and the techniques for drug application and recording, see AXELSSON and THESLEFF 1959 and THESLEFF 1960.) As ACh was released by a current pulse from the micropipette, that part of the muscle membrane at which the drug produced a depolarization, with a latency of less than 10 msec., was considered sensitive to the drug. The length of the sensitive region was measured with a binocular microscope with an eye piece scale at 80 × magnification. To avoid the use of muscle fibres in which the motor nerve might have degenerated as a consequence of the operative procedure, the end-plate region was always located and examined for the presence of miniature end-plate potentials (m.e.p.p.). The m.e.p.p. were photographed on single sweeps, and their mean frequency calculated from at least fifty m.e.p.p. from each fiber.

Since a close correlation has been shown to exist between the size of the ACh-sensitive area in a muscle, and the magnitude of contracture which is produced by diffusely applied ACh (LUNDQVIST and THESLEFF 1960), each of the muscles examined by the micro-electrode technique was subsequently studied to determine its isotonic shortening in response to graded doses of ACh. One to four weeks after the operation both tenuissimus muscles were removed from each cat, then tested for their sensitivity to applied ACh.



Fig. 1. Motor unit potentials recorded from the hindleg of cat 18 days after lower motor neurone isolation procedure. The time marking is 4 msec.

Results

Immediately following the surgical isolation of the cord segments, both hindlegs were completely inactivated as shown by electromyography and the obvious observation of paralysis. After one week of inactivity however spontaneous intermittent fasciculations were observed, and they were identified as motor unit potentials (Fig. 1). This spontaneous motor neurone activity persisted throughout the remainder of the observation period, *i. e.*, up to three weeks.

The frequency of m. e. p. p. *s* was relatively unaffected by the isolation of the segmental lower motor neurones (Table I). Neither was their amplitude nor time course altered.

When the length of the ACh-sensitive surface in a single muscle fibre is determined by iontophoretic micro-application of the drug, records such as those in Fig. 2 are obtained. The highest sensitivity is observed at the visible end-plate region (records D and E) and from that point the local sensitivity falls rather symmetrically toward both ends. For example, in Fig. 2 the total length of the muscle fibre which responds to ACh is 0.5 mm. The mean lengths of ACh-sensitivity in control muscles and in muscles with isolated motor neurones are shown in the last column of Table I. It is evident that the length of the fibre sensitive to ACh is not markedly increased by one week of complete inactivation. After a longer period, a moderate increase of the ACh-sensitive

Table I. Mean values and standard deviations for the frequency of m.e.p.p. and the length of the ACh-sensitive surface in single fibres of cat tenuissimus muscle. The values are recorded according to the duration of the period of muscle inactivity produced by surgical isolation of the motor neurones

Duration of inactivity (days)	Number of muscles	Number of end-plates	Mean frequency of m. e. p. p. per sec	Mean length of receptive surface (mm)
Control	6	35	14.3 1.18	0.67 ± 0.019
7	6	30	7.4 ± 0.80	0.81 ± 0.027
12—13	6	29	3.3 ± 0.68	(1.16) range 0.5—> 2.5
18—27	6	24	22.1 ± 3.00	(1.01)* range 0.5—> 2.5

* For technical reasons distances longer than 2.5 mm could not be measured.

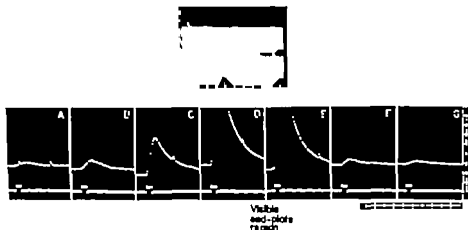


Fig. 2. Records illustrating how the length of the ACh-sensitive surface in a single muscle fibre is determined by iontophoretic release of the drug from a micropipette (currents through the pipette are registered in the lower tracing), and by intracellular recording of the resultant membrane depolarization (upper tracing). The records were obtained when ACh was released close to the fibre membrane at points increasing by 0.1 mm increments from the visible end-plate region. The m. e. p. p. frequency was recorded on successive single sweeps as shown in the upper record. The time marking is 20 msec; voltage calibration, 1 mV; monitor calibration (voltage scale) 2×10^{-4} A.

area occurred and, as shown by the range of measurements, the chemically sensitive length of a fibre occasionally exceeded 2.5 mm (for technical reasons distances longer than 2.5 mm could not be measured).

A muscle in which the chemically sensitive surface is enlarged will respond to diffusely applied ACh with a contracture (AXELSSON and THESLEFF 1959; ELMQVIST and THESLEFF 1960). ACh produced an isotonic shortening which, when the period of motor neurone isolation had lasted longer than one week,

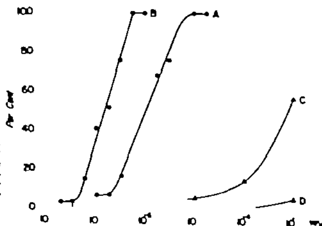


Fig. 3. Dose-response curves for ACh obtained in the cat tenuissimus. The isotonic shortening produced in the muscle by diffusely applied ACh is expressed in per cent of the shortening caused by rapid immersion of the preparation in isotonic K_2SO_4 solution. Curve A was obtained 14 days after motor denervation; B, 34 days after botulinum intoxication; C, 13 days after isolation of lower motor neurones; D after sham operation (control).

was 20—50 per cent of that observed in a chronically denervated, or botulinum intoxicated, muscle (Fig 3) However the relative ACh-sensitivity of the membrane of the inactivated muscle was much less than that following denervation. That is shown by the shift of the dose-response curve toward the right in Fig 3

Discussion

Surgical technique was used which permitted isolation of the lower motor neurones without damaging them (TOWER 1937 a b, ECCLES 1944) During the first post-operative week no sign of motor neurone activity was recorded. Subsequently however the motor neurones spontaneously became active, producing intermittent fasciculations in both hindlegs. Thus, the muscles may be considered to have been completely inactive during the first post-operative week, but later some spontaneous motor activity was present

Following motor nerve *degeneration in the cat* the spread of the receptor surface from the end-plate region toward the tendons reaches its maximum extent by covering the entire fibre within seven days (AXELSSON and THESLEFF 1959) The present investigation has shown that complete inactivation of a muscle with an intact motor unit for the same period of time causes little increase in the size of the ACh-sensitive surface of the end-plate. Therefore inactivity alone cannot be responsible for extension of the receptor surface after nerve degeneration. MILEDI (1960) has reached the same conclusion while working with incompletely denervated frog muscle. In sartorius fibres, with at least two end-plates, he observed that section of the nerve to one of them caused a spread of chemical sensitivity in the region of the latter end-plate despite the fact that impulse transmission continued through the intact junction.

However if lack of transmitter release were primarily responsible for the increase in ACh-sensitivity in chronically denervated muscle (THESLEFF 1960 b) the absence of a change following inactivity is understandable Isolation of lower motor neurones abolishes synchronous transmitter release by nerve impulses, yet leaves the spontaneous transmitter activity (i e the m. e p. p. s) intact. The continuous subthreshold transmitter release may be sufficient to maintain the chemical sensitivity of the muscle in about its normal area and intensity

The main factor causing the increased sensitivity of denervated muscle to diffusely applied ACh appears to be an increase in the chemically sensitive membrane area (AXELSSON and THESLEFF 1959 MILEDI 1960 The slight enlargement of the ACh-sensitive surface which is observed after prolonged inactivation may therefore provide an explanation for the rather moderate tenfold) supersensitivity to intra-arterial injections of ACh observed after transection of the lower thoracic portion of the spinal cord (CANNON and HAIMOVICI 1939 SOLANDT and MAGLADERY 1942)

We wish to thank Dr BIRGITTA HOLMQVIST of the Institute of Physiology for showing us the methods for the isolation procedure. This investigation was aided in part by United States Public Health Service research grant B-2646 from the National Institute of Neurological Diseases and Blindness, and by grants from the Muscular Dystrophy Associations of America, Inc., and the Air Research and Development Command, United States Air Force, through its European Office. Unfailing technical assistance continued to be provided by Miss E. ADLER.

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Influence of Noradrenaline on Spinal Interneuron Activity

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Abstract

Skoglund, C. R. *Influence of noradrenaline on spinal interneuron activity* Acta physiol. scand. 1961 51 142—149 — The effects of intra-arterial and intravenous injections of noradrenaline on spinal interneurons have been studied on decapitate, curarized cats by means of intracellular technique. The most common effect, observed at a threshold dose of 1 μg per kg bodyweight, was an increase in excitability Interneuron responses to single afferent volleys were facilitated, typical effect being transformation of single spik responses into repetitive discharges. In the absence of any induced afferent stimulation noradrenaline injec tions were observed to set up discharges in initially silent cells and to cause an increase in frequency of prevailing activity sometimes followed by a decrease. An initial decrease in excitability was only occasionally seen.

Introduction

The influence of adrenaline on spinal reflexes was shown by Schweitzer and Wright in 1937 and their finding of a modifying action of adrenaline on central transmission processes has since been confirmed in several investigations (cf. Paton 1958) After the demonstration of noradrenaline as a normal constituent in the animal body it was only natural that interest should be directed toward the possible action of this substance on the central nervous system and, as appears from Euler's review (1956) this problem has been approached along several lines. In general similar effects have been found as for adrenaline. Spinal reflexes in the decapitate cat were thus found to be influenced in the same way by noradrenaline as by adrenaline the most common effect being a facilitation of monosynaptic extensor reflexes, which appeared at about the same threshold doses for both substances when injected intra-arterially (Bernhard and Skoglund 1953 cf Skoglund 1952) In a study of the functional

properties of spinal interneurons (KOLMODIN and SKOGLUND 1954) noradrenaline was observed to influence the discharge rhythm of interneurons in the ventral horn. CURTIS *et al.* (1957) using narcotized and fully deafferentated cat preparations, only occasionally observed potentiating effects of noradrenaline (or adrenaline) on monosynaptic reflexes and also reported that none of these substances had any effect on spinal interneurons of the Renshaw type.

A more detailed analysis of the noradrenaline effects observed in non-anesthetized cats has since been made and a brief description of the modifying action on interneuron activity seemed justified, particularly in view of some recent observations of a modulating action of noradrenaline on unit activity in the reticular formation (BRADLEY and MOLLICA 1958) and at the receptor level (LOEWENSTEIN 1956)

Methods

Decapitate, slightly curarized cats (average weight 2.8 kg) kept on artificial ventilation have been used. Laminectomy was performed in the lumbosacral region and the ventral roots L_6–S were usually cut to permit antidromic stimulation. In some of the preparations dorsal roots or peripheral nerves were prepared for electrical stimulation, while other preparations were kept as intact as possible for the purpose of studying interneuron activity under as normal conditions as possible. The intravenous injections were made into the jugular vein; for intra-arterial injections the method of HOLMSTEDT and SKOGLUND (1953) using polyethylene catheter inserted in the aorta, was applied.

In most experiments Macrodex infusions were made to maintain the blood pressure occasional checkings of the systemic arterial pressure gave values between 90 and 120 mm Hg I preliminary series of experiments the blood flow in the spinal cord was studied by means of thermocouple inserted in the lumbar cord.

Capillary microelectrodes filled with 2.7—3.0 M KCl solution were used in connection with an input stage of cathode follower type. The main direct coupled amplifier connected to the oscilloscope, also fed an inkwriter for continuous recording of the membrane potential. Part of the analysis of spike frequency variations was made on tape recordings from the actual experiments (for detailed description of the recording technique see HAAPANEN *et al.* 1958)

The technical difficulties encountered in microrecording from central nervous structures when injecting substances with effects on circulation are obvious. The fact that even minute movements of the cord tissue in relation to the electrode tip are reflected in alterations in the recorded membrane potential level leads to several kinds of difficulties. Thus, it was found that injection of a few ml of Ringer solution into the aorta may result in abrupt changes in the recorded membrane potential, apparently due to tissue dislocation caused by changes in the regional arterial pressure. With intravenous Ringer injections such changes were only occasionally observed. This type of artifact is recognized by its appearance immediately after the injection. The specific blood pressure changes due to noradrenaline develop more slowly and may result in later occurring dislocation effects. In certain cases abrupt or more slowly developing changes occurred in the membrane potential level at latencies varying from 10 to 30 sec after the intravenous injections and it is likely that these were secondary to blood pressure changes, the maximum rise of which occurs within these time periods. In such cases the membrane potential could often be restored to its initial level by slight tap on the electrode holder. Variations in the recorded membrane potential due to dislocation do not influence the discharge frequency if the injury of the cell is not too large (cf. HAAPANEN *et al.* 1958

Results

For the testing of noradrenaline effects interneurons were selected that had shown a steady membrane potential and constant activity level or unchanged responses to stimulation during a sufficiently long period (usually 5 min) to allow a safe estimation of the relatively slowly developing effects. The twenty five interneurons thus selected represented cell types varying both with respect to functional connections and anatomical location.

In spite of the heterogeneousness of the cell population tested the effects of an increased noradrenaline content of the blood — whether caused by intra arterial or intravenous injections — were fairly uniform, the most commonly observed initial result being *an increase of excitability* The threshold dose of intravenous injection was 3 μg, corresponding to about 1 μg per kg bodyweight. More marked effects were obtained by doses of 2—3 μg/kg Injections of still higher doses were generally avoided because of the dislocation effects (cf. above) With intra arterial injections of corresponding doses the effects were generally more pronounced.

In one series of experiments the noradrenaline effects were tested on the interneuron response to single shock stimulation of afferent nerves and various types of facilitation effects could be observed. Thus in one preparation, in which interneurons in the ventral horn were studied, a cell was observed which initially responded to an afferent volley in the gastrocnemius nerve with a typical single spike at threshold and maximally two spikes at suprathreshold stimulation. Some minute after intravenous injection of 5 μg noradrenaline this response changed into a train of 4—5 spikes, which effect slowly subsided during the following five minutes of observation of the cell. Another cell tested responded initially to the afferent muscle nerve volley only under simultaneous facilitatory influence from a skin area. After noradrenaline the cell responded readily to muscle afferent stimulation alone. Another manifestation of an increased excitability was a greater constancy of the response at threshold stimulation observed in certain cells.

The majority of the observations were made on cells in the absence of any afferent stimulation. Under such conditions some cells are initially silent, while others show a continuous discharge. In cells of the first type noradrenaline injection was often observed to initiate discharges which varied from trains of impulses of some seconds length up to several minutes continuous firing The impulse frequency kept within the range of the naturally occurring discharges, and high-frequency bursts of injury type were never recorded Most of the cells of the second type showing spontaneous discharges from the beginning, were also found to be influenced by noradrenaline In some cases only slight effects could be observed, in the form of a change to a more irregular rhythm without any more pronounced variations in the average frequency but in others definite changes in discharge frequency could be demonstrated. The initial

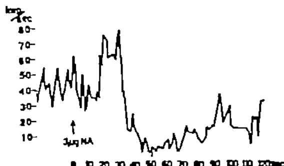


Fig. 1. Effect of noradrenaline on discharge frequency of spinal interneuron. Time in sec from moment of intra-arterial injection (at arrow).

change was usually an increase in frequency and a slowing-down of the rhythm was only occasionally observed as an immediate effect. A depressive effect may also follow the initial facilitatory phase. A typical example of such a frequency modulation of interneuron activity is given in Fig. 1. The cell had first been under observation for a period of 10 minutes, during which it had been discharging at an average frequency of 45 per sec, with maximal fluctuations between 30 and 55 per sec. An intra-arterial injection of 3 µg noradrenaline resulted in a definite increase in frequency up to 75—80 per sec. As appears from the figure, this period of increased activity lasting some 15 sec, was followed by a period of decreased frequency from which the cell slowly recovered during the second minute after the injection.

Some of the units tested were unaffected by noradrenaline. However, on the basis of the present rather limited material it is not possible to judge whether the variations in noradrenaline effects are due to different reactions of various cell types. Adrenaline was tried only in a few instances but also seemed to have a facilitatory action.

In several cases a small but definite decrease of the membrane potential was recorded simultaneously with the increase in discharge frequency and this may represent a true membrane potential change (cf. KOLMODIN and SKOGLUND 1958). However, in most cases there was no direct relationship between potential level and frequency through the whole sequence of variations induced by an injection of noradrenaline. An intermingling of dislocation effects (cf. Methods) and true membrane potential changes is the most obvious explanation of such discrepancies, and before artifact changes can be safely excluded nothing can be deduced about a possible specific membrane effect of noradrenaline.

Discussion

The results described above imply that injections in non-anesthetized cat preparations of relatively small doses of noradrenaline — 1 µg per kg body weight — are reflected in changes of single interneuron activity. For comparison, the normal noradrenaline content of the blood is about 1 µg per litre blood

plasma and the average resting secretion in chloralose-anesthetized cats has been found to be about 0.4 μg per kg bodyweight per minute, calculated on both adrenal glands (EULER and FOLKOW 1953). As an example of activity secretion may be taken the average value obtained by EULER and FOLKOW during splanchnic stimulation, 1 μg/kg/min per adrenal gland. The blood content variations brought about by the present experiments may thus be said to approach the physiological range, although such sudden changes as those induced by injections do not occur normally.

For reasons given below, the interpretation of the results as being due to a direct action of noradrenaline on the nerve cells is the most likely; however, in view of the experimental conditions this conclusion can be reached only by excluding certain indirect modes of action, well-known from similar argumentations by previous investigators in this field. Thus, since a comparatively intact preparation was used in the present experiments, so as to be able to study interneuron activity under conditions as normal as possible (cf. KOLMODIN 1957), changes in afferent inflow may be an interfering factor. HUNT's (1960) finding that discharges from muscle spindles are influenced by sympathetic hormones indicates one possible source of error of this kind, but this mechanism seems less probable, if one may judge from the large doses of intravenously injected adrenaline, 40—80 μg/kg, which were necessary to produce significant changes in the afferent inflow. WILSON's (1956) attempts to demonstrate any changes in the afferent inflow in the dorsal roots of cats after adrenaline injections were also negative.

That circulatory changes due to the blood pressure variations induced by adrenergic substances constitute a significant factor in their action on central nervous activity has been rejected by most authors (e. g. SCHWEITZER and WRIGHT 1937, WILSON 1956, BRADLEY and MOLLICA 1958). KIRWEL and DOMINO (1957) reported that the effects of adrenergic substances on the patellar reflex were maintained after stabilization of the blood pressure by means of a reservoir system and also showed that a mechanical elevation of the pressure failed to reproduce the effects. However, it should be pointed out that if the systemic arterial pressure is abnormally low from the beginning, as may be the case in decapitate preparations when necessary precautions to maintain the blood pressure have not been taken, the spinal neurons may be apt to respond to an improved circulation (cf. KOLMODIN 1957). VOORHOEVE (1960) considered the adrenaline and noradrenaline effects observed on fusiform motoneurons in decapitate cats to be due to an increase in spinal cord perfusion, since effects were only occasionally observed in decerebrate cats with arterial pressure above the critical level.

Regional changes in spinal cord circulation have also to be taken into account. As appears from SOKOLOFF's (1959) review several studies of noradrenaline effects on brain circulation have been performed, some of which indicate that the cerebral vessels are constricted to a higher degree than the over

all circulatory bed, but corresponding investigations on spinal cord seem to be lacking. FIELD *et al.* (1951) using thermocouple technique for detection of blood flow changes in the rabbit spinal cord, observed however that active vasoconstriction in the lumbar cord may result from adrenaline injections. In a preliminary series of experiments on decapitate cats, using the same technique, similar effects have been observed after noradrenaline injections. Of special interest was the finding that after intra arterial injection regional blood flow changes in the spinal cord could be observed in the absence of any more pronounced systemic effects (SKOGLUND, unpublished observations). To be able to judge of a possible vascular influence it seems that the excitatory effects should rather be correlated to regional blood flow changes than to variations in systemic arterial pressure. The relatively long latencies of the facilitatory effects of noradrenaline might point to a secondary action via regional vascular changes, but a slow reaction of the nerve cells to noradrenaline is also a possible explanation, particularly if metabolic changes are involved (cf. below).

In strong support of a direct action of noradrenaline on the nerve cells speak the findings of similar effects at the receptor level. As demonstrated by LOEWENSTEIN (1956) the facilitatory action of sympathetic stimulation on the frog's touch receptors can be reproduced by application of noradrenaline or adrenaline, and the similarities between the effects on these receptors and on the spinal interneurons are striking. Thus a touch receptor which ordinarily fired only one impulse in response to a mechanical stimulus discharged repetitively after application of sympathetic hormones, and in the absence of stimulation spontaneous firing could be initiated or increased.

However, from such analogies no conclusion should be drawn as to a transmitter function of noradrenaline in the central nervous system such as postulated at sympathetic nerve endings. It might rather be suggested that noradrenaline, as well as adrenaline, constitutes one factor relevant to the excitability level in nerve cells and other excitable structures. With respect to adrenaline some recent results seem to indicate that such an action may occur both via an influence on membrane permeability and via metabolic changes (cf. AXELSSON *et al.* 1959). The latter alternative implies that the direct action of sympathetic hormones and their secondary effects due to vascular changes may be intimately related.

The reactions of spinal interneurons to an increased noradrenaline content in the blood are strikingly similar to those observed in units of the reticular formation (BONVALLET *et al.* 1956, BRADLEY and MOLLICA 1958) and it is possible that many types of central neurons show such sensitivity although to a varying degree. A further analysis of the action of sympathetic hormones on central neurons is of special interest in view of the fact that the secretion from the suprarenals may be increased under certain conditions. In asphyxia, a considerable increase has thus been observed (RAPELA and HOUSSAY 1952 a) and it is obvious that this finding should be taken into consideration in the inter

pretation of frequency changes seen in spinal interneurons (KOLMODIN and SKOGLUND 1959) as well as in cortical neurons (CREUTZFELDT *et al* 1957) during asphyxiation. Knowledge of the action of sympathetic amines *per se* on interneurons is, in fact, also a necessary basis for the analysis of the central action of certain drugs, such as nicotine, which is known to cause a greatly augmented adrenal secretion (RAPELA and HOUSSAY 1952 b, FOLKOW and EULER 1954)

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Renal Clearance in Dogs with Regard to Variations According to Age and Sex

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Abstract

Åsheim, A., F. Persson and S. Persson. *Renal clearance in dogs with regard to variations according to age and sex.* Acta physiol. scand. 1961. *51.* 150—162. — Repeated determinations of renal clearance of inulin and *PAH* were done in 32 dogs. Statistical analysis showed no variations with age and sex. Repeated determinations of Tm_{PAH} were done in 16 dogs. The results indicated that even under standardized conditions Tm_{PAH} is highly varying. Statistical data are presented to show that the so-called self-depression begins to occur even at the lowest plasma *PAH*-concentrations considered necessary for achieving sufficient tubular load.

In most of the published investigations on renal clearance in dogs the normal values given are based upon small series of observation. There are only a few reports on statistically treated results of studies on large series. Hou x (1948) presents data on glomerular filtration (GF) and effective renal plasma flow (RPF) in 75 normal dogs. Kubicek *et al.* (1953) reports similar results for 32 dogs. Kolberg (1959) quotes mean values for GF, RPF and Tm_{PAH} (maximal tubular excretion capacity for para amino-hippuric acid) in 11 dogs. All the said authors used female dogs. In the available literature only one publication is found in which male dogs were used in clearance studies, namely that by Stamler, Katz and Rodbard (1949). They report experiments with six male dogs and give the mean value for GF and RPF but no *Tm*-determinations were done.

In all the above mentioned studies, excepting those of Kolberg, only creatinine was used for measuring glomerular filtration rate. Kolberg gives results of inulin clearance as well.

As regards the determination of Tm_{PAH} in dogs, published data are very sparse. SMITH (1951) gives mean values for Tm_{PAH} calculated from results in various reports on small series of observations. KOLBERG (1959) gives the mean value for 11 dogs. No reports concerning Tm_{PAH} in male dogs can be found in the literature.

In the above-mentioned results of Tm_{PAH}-determinations the so-called self-depression was not taken into consideration, and, therefore, the quoted mean values may be suspected to be impaired by serious errors. This will be discussed later.

By means of a clearance technique, earlier authors have shown that renal function in children is fully developed at a very early age (WEST, SMITH and CHASIS 1948). Similar studies in young dogs have not been published.

In the work presented here normal values for GF and RPF measured by inulin-clearance and *PAH*-clearance are given. In the statistical analysis variations in age and sex were taken into consideration. Determinations of Tm_{PAH} were also done and the value of such determinations in dogs is discussed.

Material

The experimental series consisted of 32 normal dogs, 21 females and 11 males. Out of these 16 were mongrels and 16 cocker spaniels from five different litters. The latter were presumed to have an inherited disposition to hypoplasia of the renal cortex. Since the symptoms of this disease usually appear before the age of 1 / year renal function was studied continuously over this period of time. Only dogs without manifestations of the disease were included in this material. Besides clearance determinations, periodical checkings were done which as regards the urine included specific gravity, protein, glucose, bilirubin, haemoglobin and sediment. The blood examinations comprised red-cell count, total white-cell count, erythrocyte-sedimentation rate, haemoglobin estimation, haematocrit, and non-protein nitrogen determination. The serum-level of electrolytes and cholesterol was also followed. ECG, intravenous urography and measurement of the intra-arterial pressure were carried out repeatedly. Eleven of these 16 dogs were subjected to autopsy after killing; the post-mortem examinations gave negative results. From another experimental series further normal values were calculated, but these will not be reported here so as to avoid heterogeneity.

Methods

Clearance determinations

Before each clearance observation the dogs were fasted for about [illegible]0 hours. This was done to obviate those variations in GF and RPF which are considered to occur at intake of protein. In the first few experiments 500 ml of water were given by the conventional method orally [illegible] hour before the start of the observation to ensure satisfactory diuresis. The result of this was, however, that urinary output fell during the course of the experiment, and, hence, the influence of the "dead space" in the kidney was likely to increase. The clearance-values from these first experiments were, however, in full agreement with those obtained later, when the dogs were allowed water *ad lib.* Diuresis thus became even, and together with steady blood-levels of inulin and *PAH* the experimental conditions may be considered more standardized. Diuresis was as a rule moderate and was

never below 0 10 ml per minute. It should be noted here that the experiments included small dogs, too. Body-weight ranged from 7 to 16 kg.

The dogs were anesthetized with mebumal[1] given intravenously and since the observations were done in undisturbed and quiet surroundings, they could be kept in very light sleep right through the experiment. A Maghill's tube was used for intubation. A polyethylene catheter of conventional type was inserted into the cephalic vein and into the femoral artery (Helander, Asheim and Odman 1958) The vein catheter was used for giving the infusion, and the one in the artery for withdrawing specimens of blood and also for measuring the blood-pressure by connecting it with a mercury manometer The initial dose of *PAH* and inulin was given, approximately 15 mg and 40 mg per kg body-weight, respectively Continuous infusion of *PAH* and inulin was then started immediately by means of an electrically driven infusion apparatus. The concentration of *PAH* in blood plasma was maintained as close to 2 mg per 100 ml as possible, and the concentration of inulin between 15 and 50 mg per 100 ml. After a 45 min interval of equilibration the first clearance period was begun after the bladder had been emptied through a Foley catheter of suitable size. The evacuation of the bladder was aided by manual pressure on the abdomen over the bladder and by rinsing with physiological saline solution and a final rinsing with air The urine was collected over a 20 min period. Blood was withdrawn from the femoral artery in the middle of the period. The heparinized centrifuge tube with the blood was immediately placed in a container with ice-water where it was allowed to stand for about 5 min before centrifuging. Three such consecutive 20 min periods were completed. *Tm*-determinations were then made immediately A primary dose of about 200 mg of *PAH* per kg body-weight was given by very slow injection. Continuous infusion of *PAH* and inulin was started. As far as possible a plasma-level of *PAH* exceeding 15 mg per 100 ml was maintained. The concentration of inulin was kept at the same level as earlier After a 30 min equilibration interval two 20 min periods were as rule completed.

Analytical methods for determination of the clearance agents

Josephson and Godin's (1943) modification of Corcoran and Page's (1939) method was used in the inulin analyses. *PAH* was determined by the method of Smith (1951) All the analyses were carried out with Beckman spectrophotometer model B. Duplicate determinations were made of the inulin and *PAH* concentrations in each specimen of blood and urine and the means were calculated.

Results

A. Glomerular filtration rate and effective renal plasma flow

The results for the 16 cocker spaniels are given in Table I The total number of clearances is 54 with approximately 162 observations. The values for both GF and RPF are expressed per kg body-weight and per sq m body surface (Cowgill and Drabkin 1927)

[1] The formula of mebumal is as follows

5-ethyl-5(1-methylbutyl)-malonylcarbamide (Pentobarbitone)	1.8 g
Pentobarbitone sodium	4.0 g
Urethane	25.0 g
Spir conc.	15.0 g
Glycerin	12.5 g
Aq. steril.	ad 100 ml

Table I Clearance of inulin and PAH in 16 cocker spaniels. Each value is the average of 1 to 4 observations, each of which in turn is the average of 3 consecutive periods

Dog	Sex	Filtration rate (C_{In}) ml/min.		Renal plasma flow (C_{PAH}) ml/min.		Filtration Fraction C_{In}/C_{PAH}
		per kg	per m²	per kg	per m²	
I 2	♀	4.33	89	15.85	325	0.28
I 3	♀	3.92	88	11.98	270	0.30
I 4	♀	3.18	68	10.47	218	0.31
II 1	♀	4.49	96	12.57	267	0.36
II 2	♀	3.64	74	16.91	350	0.24
II 3	♀	3.05	67	10.88	242	0.28
II 5	♂	3.88	91	12.63	301	0.30
III 1	♂	4.16	90	12.24	324	0.28
IV 1	♀	3.12	61	12.67	249	0.26
IV 2	♀	3.59	72	14.34	288	0.28
IV 3	♂	4.21	91	17.83	389	0.24
V 2	♂	3.71	80	13.71	294	0.27
V 3	♀	3.84	70	15.77	298	0.25
V 4	♂	5.86	100	17.71	301	0.33
V 5	♂	2.94	54	9.03	167	0.32
V 6	♀	3.47	62	14.68	258	0.33

Since the values in Table I represent observations on dogs of various ages and of both sexes, it was considered necessary to study any presence of significant variation of the values with age and sex before treating the whole material statistically. The results from the clearance tests arranged in age-groups, showed no tendency to variation with age within the range concerned.

For calculation of any variation with sex the *t*-test was used. The mean values for C_{In} and C_{PAH} calculated from values given in Table I showed no significant differences.

Both for C_{PAH} and for C_{In} $P > 0.05$. The *t*-value for C_{In}, however, is as great as 1.177.

Since glomerular filtration is reported to be higher in men than in women, and since our material comprises only 6 male dogs, the series was augmented by 16 mongrel dogs, including 6 males. The age of these 16 dogs corresponded to that of the rest of the series. The values for this additional material are given in Table II arranged as Table I.

In consideration of this larger material ($n = 32$) (Table I and Table II) with respect to sex difference it will be seen that there is no significant deviation either for C_I ($0.3 > P > 0.2$) or for C_{PAH} ($0.2 > P > 0.1$). The mean values for C_{In} are for male dogs 81 ± 4.9 ($n = 11$) and for female dogs 71 ± 3.1 ($n = 21$). The mean values for C_{PAH} are for male dogs 28[illegible] ± 24.3 ($n = 11$) and for female dogs 251 ± 10.5 ($n = 21$).

Table II Clearance values from 16 mongrel dogs

Dog	Sex	Filtration rate (C_{In}) ml/min.		Renal plasma flow (C_{PAH}) ml/min.		Filtration fraction C_{In}/C_{PAH}
		per kg	per m²	per kg	per m²	
1	♀	2.72	63	9.44	220	0.29
2	♂	3.50	75	11.00	236	0.32
H 2	♀	4.66	102	14.60	321	0.32
H 3	♀	4.43	75	11.29	193	0.38
H 5	♀	3.50	67	10.20	196	0.35
H 6	♀	4.10	79	13.10	251	0.31
H 7	♀	4.71	80	10.86	185	0.44
H 8	♀	4.33	80	11.67	216	0.38
H: 9	♂	4.36	86	21.18	419	0.26
H: 10	♀	4.70	90	14.70	282	0.32
H: 11	♀	4.57	78	17.00	289	0.26
H 26	♂	2.64	61	7.64	175	0.34
H: Gr	♀	1.74	44	6.50	160	0.27
H St		3.11	71	9.88	226	0.32
H Sc	♀	4.11	94	12.82	293	0.32
H: Co	♀	2.20	42	11.30	217	0.19

Since there seems to be no significant variations with repect to sex or age the data concerning C_{In}, C_{PAH} and FF (filtration fraction, C_{In}/C_{PAH}) for the whole material have been analysed statistically and the results appear in Table III.

Skewness of the distribution was estimated by Lindeberg's formula

$$S_L = P - 50,$$

here P is the percentage of the total number of observations whose value exceeds the mean. The standard error ΣS_L for S_L was calculated from the formula

$$\Sigma S_L = \pm \frac{30}{\sqrt{n}}$$

and with Student's t-test the following was obtained

$S_L \pm \Sigma S_L$ for

C_I/kg	3.12 ± 5.30	$(0.6 > P > 0.5)$
C_{In}/m	0 ± 5.30	$(P = 1)$
C_{PAH}/kg	-9.38 ± 5.30	$(0.1 > P > 0.05)$
C_{PAH}/m	3.13 ± 5.30	$(0.6 > P > 0.5)$
FF	-3.12 ± 5.30	$(0.6 > P > 0.5)$

It will be seen from these calculations that the distributions can be regarded as normal.

Table III Statistical analysis of filtration rate and effective renal plasma flow in normal dogs

	C_{In} ml/min.		C_{PAH} ml/min.		Filtration fraction
	per kg	per m²	per kg	per m²	C_{In}/C_{PAH}
	No. of dogs 22				
Mean	3.77	76	12.88	263	0.31
Range	1.74—5.86	42—102	6.30—21.18	160—419	0.19—0.44
Standard deviation	0.84	15	3.21	62	0.051
Standard error	0.15	2.69	0.57	11.17	0.0089
Coefficient of variation.	22.2	19.8	24.9	23.6	16.5

B Max renal tubular excretion capacity for PAH

By subtracting the amount of *PAH* which is filtered through the glomeruli from the amount excreted in the urine per unit of time a measure is obtained of the amount of *PAH* actively excreted via the tubules. This can also be expressed by the formula (SMITH 1951)

$$T_{PAH} = U_{PAH} \times V - C_{In} P_{PAH} FW$$

where the symbols denote conventional values. In dogs *FW* is considered to be constant and independent of the plasma concentration up to 60 mg per 100 ml and is quoted at 0.917 (SMITH 1951). In our calculations the constant 0.92 was used throughout. The results of the T_{PAH}-determinations appear in Table IV. Four negative values from two dogs are listed separately in Table V. The accumulation of *PAH* in the tubules is calculated from the formula

$$P_{PAH} \times C_{PAH} - C_{In} \times P_{PAH} \times FW$$

each expression being of conventional denotation.

Since there is great variation in the *T*-values and this variation seems to show some correlation with the blood-plasma concentration of *PAH* the values were inserted in a co-ordinate system with T_{PAH} as the ordinate and P_{PAH} and the ratio load T_{PAH} respectively as the abscissa (Fig 1 and 2). It will be seen from these two figures that there is a tendency to falling T_{PAH} with rising P_{PAH} or rising load T_{PAH}. The values given in Fig 1 were treated statistically by regression analysis (Fig 3). To find out whether the regression was linear the material was grouped in such a way that P_{PAH} was given in a precision of 5 mg/100 ml. The *T*-variable is called *y* and the designation *x* is used for the integral part of $PAH - 10$ 5. The regression analysis gives the straight line $y = 25.68 - 0.70$ and the standard deviation around the line 8.91. The present material does not show a significant deviation from a linear regression, that is, linear regression is accepted. A test for significance shows that the regression coefficient diverges significantly from 0, that is, hypothesis of a negatively inclined straight regression line is accepted.

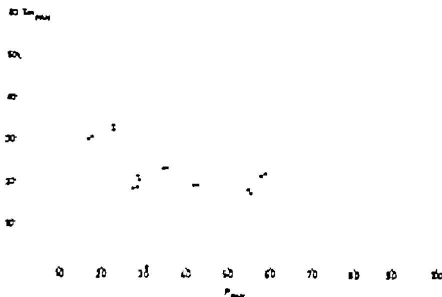


Fig 1 One hundred Tm_{PAH}-determinations with various plasma-concentrations (P_{PAH}) Tm_{PAH} is given in mg/min/m² and P_{PAH} in mg/100 ml.

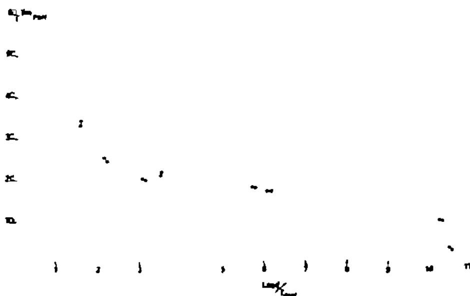


Fig. 2. The same Tm_{PAH}-determinations as in fig. 1 now in regard to the ratio load, T_{PAH}. The six lowest values for Tm_{PAH} (highest load T_{PAH}) are not included. Tm_{PAH} is given in mg min m

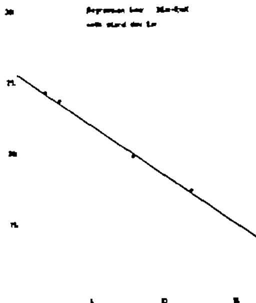


Fig. 3. Regression of y on x, where y is Tm, given in mg/min/m and is the integral part of $\frac{PAH - 10}{5}$. P_{PAH} is given in mg/100 ml.

To enable a comparison between our results and those given by different authors we have calculated T_{PAH} for those values which were obtained at a tubular load/T_{PAH} between 1.5 and 4 0. For 46 such observations the mean value for T_{PAH} is 25 43 with standard deviation 7 04 mg per minut per sq m, or 1.21 with the standard deviation 0.34 mg/min/kg

Table V records the afore-mentioned negative T values. The possibility that these are due to some analytical error cannot be excluded, although there is nothing to suggest such an error The occurrence of negative T-values would mean that some part of the filtrated PAH as well is reabsorbed. Schachter and Freinkel's theory that self depression would be due to reversible inter ferences with the mechanism of transport in the t bules is, however supported by MUDGE and TAGGART (1950) who by dministration of acetat were able to control this self-depression and raise the maximal tubular excretion. Since the T_{PAH}-determinations reported here were done n cocker spaniels, the question arises as to whether this breed reacts n a specific way In a series of 16 dogs of v rious breeds, however th results of 23 Tm-determinations at various plasma le ls of PAH were analogous to those reported for cocker spaniels.

Discussion

Anesthetized dogs were used n the studies. Deep anesthesia for a prolonged period may probably depress ertain bod ly funct ons, uch as temperatur blood-pressure and, as a result of th altered blood pressur enal function as w ll.

Table IV T_{PAH} *determinations in correlation to plasma-concentration* (P_{PAH}) *and rate load* T_{PAH}. T_{PAH} *is given as mg/min/m²* *and* P_{PAH} *as mg/100 ml. Number of observations = 100*

Dog	T_{PAH}	P_{PAH}	Load/T_{PAH}	Dog	T_{PAH}	P_{PAH}	Load/T_{PAH}
I 2	33.81	25.00	2.50	III 1	9.70	28.41	9.63
	30.44	34.17	1.67		6.59	25.89	9.4[illegible]
	13.86	50.94	9.01		25.21	31.57	4.31
	18.84	43.42	5.[illegible]6		19.93	28.39	4.90
	17.47	45.09	4.71		33.16	22.55	1.56
	24.05	35.07	2.59		31.29	16.70	1.21
	31.02	63.79	4.87		34.25	22.55	1.33
	20.89	52.61	7.53		33.24	16.67	1.20
I 3	33.66	33.33	1.68	IV 1	17.98	68.47	14.09
	24.64	23.53	1.56		23.22	58.45	9.73
	18.80	43.67	3.12		18.92	76.82	3.6
	15.64	35.00	3.16		20.98	63.13	4.26
	20.15	42.51	3.50		14.05	73.48	6.31
	17.64	31.67	2.93		13.24	66.80	3.09
	19.20	54.28	7.44		17.73	42.59	3.5[illegible]
	15.08	47.60	8.40		19.92	39.25	77
I 4	28.89	42.50	3.03	IV 2	30.69	30.90	2.56
	31.88	27.50	1.08		27.83	23.58	[illegible].01
	5.89	64.30	1.15		14.60	81.80	13.63
	1.38	57.78	42.01		11.41	78.50	17.67
	30.50	64.30	4.39		24.38	34.40	[illegible].37
	26.59	54.28	4.10		23.87	29.23	[illegible].05
	26.8[illegible]	89.33	5.19		15.87	37.38	3.30
	28.15	80.16	4.50		21.63	52.52	3.4[illegible]
II 1	38.55	45.05	1.12	IV 3	17.93	75.00	6.0
	12.81	34.86	3.57		16.15	69.00	5.84
	18.54	3.11	6.50		33.72	13.96	1.01
	10.96	51.77	10.2		30.71	12.53	1.49
	23.51	73.48	7.28		36.18	18.37	[illegible]
	22.96	61.79	6.93		31.89	15.03	[illegible]
II 2	22.88	57.50	5.41	V 2	23.40	15.87	1.03
	1.49	46.67	[illegible].02		20.[illegible]1	12.53	1.42
	21.07	45.93	5.08	V 3	22.03	41.75	3.46
	4.59	40.08	17.51		20.46	34.24	3.08
	19.17	30.90	5.01		34.77	32.37	[illegible].61
	24.80	15.87	2.17		28.87	27.5[illegible]	[illegible].57
	4.34	50.90	6.54		5.37	20.04	8.11
II 3	17.97	65.00	6.13		5.07	13.87	10.58
	1[illegible].99	61.6[illegible]	8.52	V 4	34.74	26.7[illegible]	1.13
	13.91	56.78	7.33		31.87	17.54	1.41
	20.11	41.72	3.91	V 5	24.35	65.13	3.23
	12.21	71.81	9.19		23.71	52.61	[illegible]
	10.62	6[illegible].97	10.30		21.[illegible]9	31.73	1.90
	22.53	39.25	3.97		19.39	27.59	1.8[illegible]
	22.32	8.39	2.80	V 6	24.19	58.45	6.18
II 5	18.52	34.33	4.38		24.09	47.09	5.[illegible]
	2[illegible].[illegible]0	24.22	3.91		25.55	36.74	[illegible].11
	18.37	20.85	2.61		21.72	29.23	1.73
	33.13	37.56	1.9[illegible]		0.74	30.90	33.33
	26.13	22.54	2.00		3.77	26.7[illegible]	10.5

Table V. Clearance values in dogs each with 2 observations which gave negative T_{PAH}

Dog	H 1		V 5	
Weight kg	11	11	11	11
Body surface area m²	0.524	0.524	0.524	0.524
Period (minutes)	1(20)	2(20)	1(15)	2(15)
Diuresis ml/min.	0.33	0.43	0.33	0.33
Urine concentration of *PAH* mg/100 ml	6,030	7,0?0	3,000	2,350
Plasma concentration of *PAH* mg/100 ml	75.15	63.46	35.07	28.59
Filtration rate C_{In} ml/min.	75	86	37	35
Renal plasma flow C_{PAH} ml/min. Mean value	157	157	79	79
T_{PAH} mg/min.	−18.6	−19.9	−2.0	−0.7

CORCORAN and PAGE (1943) state that anesthesia induced by pentobarbitone sodium does not influence the clearance of inulin and diodrast, but that a slight rise of the arterial pressure would occur. CRAIG, VISSCHER and HOUCK (1945) have studied renal function in the dog under ether and cyclopropane anesthesia. With light anesthesia renal function is not affected, while under deep anesthesia it is reversibly depressed. REMINGTON *et al.* (1949) have shown that barbiturates given i.v. produce first hypotension and then slight hypertension. SELKURT and GLAUSER (1951) consider that the reabsorption of sodium is slowed down under prolonged surgical anesthesia induced by pentobarbitone sodium.

The effect of anesthesia on renal function is thus dependent upon the depth of anesthesia. In the present experiments mebumal was used as an anesthetic. Very light sleep was induced and its depth was checked by the eye-closure and toe reflexes. Such checking will, of course, allow some subjectivity. A fact that lends support to the presumption that the anesthesia did not depress renal function is that the clearance observations were not continued for more than two hours, while in the T_m-determinations, which were always done immediately after the clearance tests, the dogs were kept anesthetized for about 3 hours. GLAUSER and SELKURT (1952) state that in deep and prolonged anesthesia barbiturates do not affect glomerular filtration but reduce total plasma flow. Accordingly, with such an effect FF should increase. In our experiments the filtration fraction was throughout very constant during the different clearance periods, and, therefore, no influence whatsoever can have interfered with the determinations of C and C_{PAH}.

GLAUSER and SELKURT have shown that barbiturate anesthesia not exceeding 3 hours does not involve any risk of depressed renal function. On the other hand WHITE (1957) and STORMEX (1958 a and b) have demonstrated a lowering of Tm_{PAH} under anesthesia induced by pentobarbitone and thiopentone sodium. The Tm_{PAH} values given by STORMEX were, however, even for unanesthetized

dogs lower than those obtained by us. In our *Tm*-determinations the anesthesia lasted for about 3 hours and was very light.

As regards the arterial blood pressure we found full agreement with the studies by REMINGTON *et al.* (1949). As has been reported in an earlier paper (ÅSHEIM, HELANDER and PERSSON 1958) the blood pressure was on several occasions also measured before the induction of anesthesia was started. No significant differences between blood pressure values before and during the anesthesia were noted.

One disadvantage of mebumal is that it contains alcohol which causes a tendency to hemolysis. To diminish hemolysis the collected blood specimens *were treated in a special way as described previously.*

The values for C_I and C_{PAH} obtained from our observations are in good agreement with values reported earlier by HOUCK (1948) and KUMICEK *et al.* (1953) among others.

Concerning *Tm*-variations in dogs the literature contains very sparse statistical data with varying results (MUDGE and TAGGART 1950, SMITH 1951, KOLBERG 1959). The results of our studies disaffirm the occurrence of truly constant Tm_{PAH}-values in dogs, even though the plasma level of *PAH* is kept within accepted limits. Instead, it will be seen that T_{PAH} approaches *0* as P_{PAH} rises. This phenomenon, in the literature referred to as self-depression, has been discussed by several authors. In cats EGGLETON and HABIB (1950) were the first to show such a depression of Tm_{PAH} at plasma-*PAH* concentrations exceeding 30 mg per 100 ml. They considered that the depression was due either to a back-diffusion in the tubules of *PAH* or to a toxic effect on the proximal tubular cells. The value of their study is somewhat lessened by the fact that their observations were made at rapidly changing plasma concentrations, which *is a well documented cause of misjudgements.* JOHNSON *et al.* (1953) have confirmed EGGLETON and HABIB's results by showing that in the cat there is a complete inhibition of the tubular excretion at plasma-concentrations of about 20 mg of *PAH* per 100 ml or 30 mg of Diodon per 100 ml. They also found negative *T*-values, which was considered to suggest a reabsorption of *PAH* and Diodon. In similar experiments in rabbits inhibition of tubular excretion was not noted. SCHACHTER and FREINKEL (1951) have demonstrated self-depression for Tm_{PAH} in non-anesthetized dogs. *They hold that self-depression can be seen* or fail to occur on different occasions in the same animal. They assume that the self-depression is due to reversible interferences with the tubular transport and not to back-diffusion or to toxic effect. They consider that self-depression occurs at a ratio of load T_{PAH} *that is higher than 6 and that the definite* Tm_{PAH} should be calculated from values obtained at load/T_{PAH} between 1.5 and 4.0. STÖREN (1958 a) in a recently published study on Tm_{PAH} in dogs, has demonstrated that self-depression occurs earlier (load T = 4) under pentobarbitone anesthesia than in nonanesthetized dogs.

BARCLAY, COOKE and DE MURALT (1950) have noted reabsorption of *PAH*

and Diodon from normal kidneys in man. Negative Tm-values in renal disease both in man and in dogs have also been reported by some authors, among others JOSEPHSON (1947). JOSEPHSON points out that EGGLETON and HABIB's results in cats can hardly be relevant to man, since such high blood-concentrations are not used for studies on man. There is no conclusive evidence of the occurrence of self-depression in man (JOSEPHSON 1947, JOSEPHSON *et al.* 1932, JOSEPHSON and EK 1958).

POULSEN (1956), in studies on cows, has observed a lowering of Tm for Diodrast, when the tubular load is increased to such an extent that the ratio load/T_D exceeds 5.

The results obtained in our studies of Tm_{PAH} indicate that the maximal active tubular excretion in dogs under the standardised experimental conditions is highly varying. We have thus confirmed SCHACHTER and FREINKEL's observations concerning self-depression. In opposition to these authors, however, we have shown that a constant Tm_{PAH} cannot be obtained even at a certain maximal plasma-concentration of *PAH*. Self-depression begins to occur even at the lowest *PAH* concentrations considered necessary for achieving a sufficient tubular load.

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Acta physiol. scand. 1961. 51 163—168

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Electromyographic Findings in Experimental Botulinum Intoxication

By

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Received 22 September 1960

Abstract

Josefsson, J.-O. and S. Thesleff. *Electromyographic findings in experimental botulinum intoxication.* Acta physiol. scand. 1961. *51* 163—168. — The electrical activity of botulinum intoxicated skeletal muscle was examined in the rabbit by the use of electromyography. 5 to 6 days after an intramuscular injection of the toxin, fibrillation potentials appeared and persisted for a period of at least 60 days. The potentials were similar in amplitude and time course to those recorded following motor nerve degeneration. Botulinum toxin, however is known not to affect the morphological structure of the motor nerve and its terminals. Its sole action is to block transmitter release selectively from cholinergic nerve endings. Our results therefore suggest that in nerve degeneration lack of transmitter release is primarily responsible for initiating the process which produces the electromyographic pattern typical of denervation.

Degeneration of a motor nerve is generally not associated with complete cessation of the electrical activity of muscle fibres. For a period after denervation, the length of which varies from species to species, there is no activity. But afterwards there occur spontaneous action potentials of short duration and low voltage (0.5—3 msec and 50—300 µV). These potentials are thought to arise from single muscle fibres, and are called *fibrillation potentials*. Generally the mechanical activity produced by fibrillations is too feeble and asynchronous to be visible.

The fibrillation activity of chronically denervated muscles is likely to be due to the absence of some influence exerted when the motor innervation is intact. The purpose of the present investigation has been to determine whether or not the release of the chemical transmitter agent provided such an influence. Use

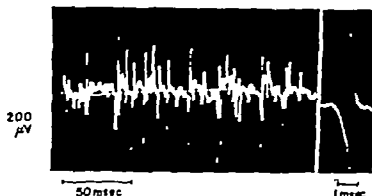


Fig. 1 Fibrillation potentials in the rabbit tibialis ant. muscle 20 days after the administration of botulinum toxin. The record on the right shows one of these potentials at faster sweep speed.

was made of botulinum toxin, which has been shown to prevent the release of acetylcholine (ACh) from cholinergic nerves without causing degeneration of the axis cylinder or the nerve terminals. (AMBACHE 1949, BURGEN, DICKENS and ZATMAN 1949, BROOKS 1956, THESLEFF 1960). It will be shown that in *botulinum intoxicated muscles there occur fibrillation potentials similar to those in denervated muscles*, and that, consequently, in nerve degeneration lack of transmitter release is to be considered responsible for the spontaneous activity of denervated muscles.

Methods

A powdered preparation of Cl. botulinum toxin type A with a mouse LD_{50} of 0.0[illegible] µg/kg was used. 1 mg of the toxin was dissolved in 1 ml of sterile phosphate buffer as described by AMBACHE (1949). Further dilutions of the toxin were made from this stock solution immediately before use. A fresh stock solution was prepared for each day experiment.

The toxin, in amounts ranging from 0.0[illegible] to 0.1 µg was injected in divided amounts into the musculature of the hind legs of 22 rabbits. With these doses and mode of administration the action of the toxin was confined mainly to the site of application. Generalized intoxications were only observed with the highest dose of the toxin. As described by GUYTON and MACDONALD 1947 and THESLEFF 1960 the neuromuscular block produced by a single dose of botulinum toxin reaches its maximum in about 5 days, and then remains at a constant level for a period of several months.

For comparison the sciatic nerve was cut in 20 rabbits, producing complete motor denervation of the hind legs. At two day intervals for a period between 3 and 8 weeks after the administration of the toxin, or the denervation procedure, the electrical activity was recorded in the hind legs by the use of electromyography.

Electromyographic recording was made by a concentric needle electrode (Disa, 2.0 k[illegible]) shunted by 15 nF at 100 [illegible]. The electrode was connected through an amplifier (Grass model P 5) to a cathode-ray oscilloscope. The potentials were photographed on single successive sweeps. The amplitude of individual potential was taken as their peak-to-peak value. The duration was the time interval between the initial deflection from the base line and the point at which the terminal deflection again returned to the base line.

In order to test the response of botulinum intoxicated muscles to ACh, a cannula was introduced into the iliac artery. Through this cannula intra-arterial injections of

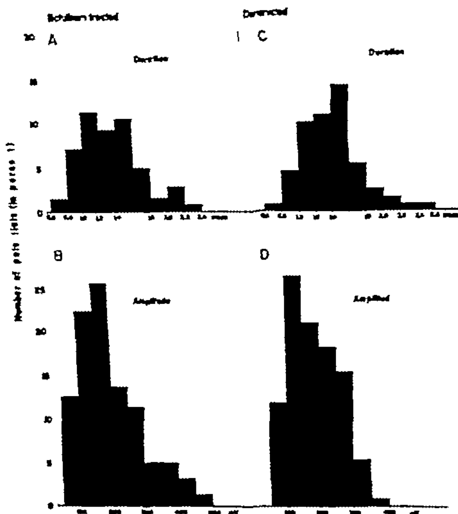


Fig. 2. Distribution of durations and voltages of potentials of the fibrillation type recorded in the tibialis ant. muscle of the rabbit 10 to 30 days after botulinum administration (A and B) or motor denervation (C and D). The graphs A and C were derived from 70 potentials; B, from 100; D, from 109.

ACh were made in a volume of 0.5 ml. Electromyographic records of the response of the tibialis anterior muscle to the injection of the drug were made simultaneously with a record of its isometric tension registered on a Grass model 5 polygraph.

Results

About 5 days after the intramuscular administration of botulinum toxin occasional action potentials of low voltage and short duration appeared. At the same time a desynchronization and a reduction of duration and amplitude of



Fig 3. Desynchronized motor unit potentials recorded from the rabbit tibialis ant. muscle 18 days after the administration of small dose of botulinum toxin.

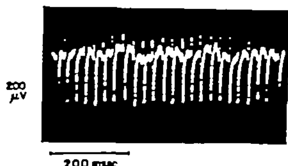


Fig 4 Repetitive potential discharge following mechanical stimuli on the insertion of the needle electrode in the rabbit tibialis ant. muscle 1 days after botulinum toxin administration

motor unit potentials occurred. After about 10 days of into ... the motor unit potentials were replaced by potentials of the fibrillati ... ig 1) and they subsequently persisted throughout the *entire observat* ... *f 10 days.* The distributions of durations and vol ... es of such pot ... en in Fig 2 A and B. To exclude the pos ... t small mot ... tials were included, the sciatic nerve was ... the recordin ... bserved that the duration of the poten ... *mum into* ... *les ran,* between 0.6 and 2.4 msec, and ... n amplit ... 1 0 μV

In instances of slight intoxica ... frequentl ... ed mot unit potentials were present (F ... e, follo *lation, bursts of single potentia* ... a also were recorded Fig 4

Animals which did not rece ... m, cutting the sciatic nerve, show ... fi the operation. Th distribution potentials are shown in Fig 2

Their mean duration and amp correspond to the 1.3 msec and potentials in botulinum intoxicat

BROWN (193 and, subsequen shown that close arterial injection malian muscle produces an electr



Fig. 5. The response of the tibialis ant. muscle to intra-arterial injection of ACh 16 days after the administration of botulinum toxin. The upper tracing shows the electrical activity of the muscle and in the lower tracing its isometric tension is recorded. The arrow shows the moment when 60 μg of ACh was injected into the iliac artery. Time scale, one sec/square.

of twitches. In an innervated muscle ACh caused muscle twitches but never a lasting increase in tension. It was therefore of interest to examine whether or not the response of a muscle chronically intoxicated with botulinum to ACh would be similar to that of denervated muscle. Fig. 5 shows the response to ACh of a tibialis anterior muscle paralysed for 16 days by botulinum toxin. When given intra-arterially the drug produced a contracture, which was accompanied by a marked reduction of the electrical activity of the muscle. The effect of ACh was thus similar to that produced in muscles following degeneration of their motor nerve.

Discussion

Characteristic of botulinum poisoning is a lack of transmitter release from cholinergic nerves, while otherwise nerve and muscle are unaffected by the toxin (AMBACHE 1949, BURGEN et al. 1949, WRIGHT 1955, BROOKS 1956, STEVENSON 1958, LAMANNA 1959). Recently KATZ (quoted by THESLEFF 1960) has demonstrated that the ultrastructure of motor nerve terminals is unaltered by the toxin. Thus degenerative changes in the nerve endings similar to those described during Wallerian degeneration (BIRKS, KATZ and MILEDI 1960) are not observed even after long-lasting and complete paralysis produced by botulinum toxin. It is consequently reasonable to assume that the toxin has a selective mode of action, and that its site of action is at the mechanism responsible for transmitter release from cholinergic nerves (THESLEFF 1960).

As shown by the present investigation, the electromyographic findings in chronic botulinum poisoning are almost identical with those found following motor denervation. Typical and almost diagnostically significant for chronic denervation are fibrillation potentials (DENNY-BROWN and PENNYBACKER 1938). These potentials were observed in botulinum intoxicated muscles. Since, as

mentioned, botulinum toxin is without effect on the morphological structure of the motor nerve and its endings, the conclusion is that in nerve degeneration lack of transmitter release is, primarily responsible for initiating the process which produces the electromyographic pattern typical of denervation.

Action potentials of the fibrillation type have been described as occurring in fetal muscle before its functional innervation (MARINACCI 1959) in adult denervated muscles, and now in muscle intoxicated with botulinum. It is interesting that in those three conditions the entire muscle membrane also has a high and uniform sensitivity to ACh (DIAMOND and MILEDI 1959 AXELSSON and THESLEFF 1959 THESLEFF 1960) It is therefore tempting to assume that the spontaneous fibrillation activity is in some way correlated with the high chemical sensitivity of the muscle fibre, but such a connexion remains to be shown experimentally

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Acta physiol. scand. 1961 51 169—174

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Cineangiocardiographic Studies of Puppies and Full-Grown Dogs

By

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Abstract

Gribbe, P. L. Hirvonen and T. Peltonen. *Cineangiocardiographic studies of puppies and full-grown dogs.* Acta physiol. scand. 1961. *51* 169—174. — Cineangiocardiographic studies have been carried out on puppies and full-grown dogs. The volumes of the left atrium and the left ventricle were calculated using 35-mm film, exposed at rate of 48 pictures per second. The stroke volume and residual blood volume were calculated from the volume curves. The stroke volume calculated as a percentage of the end-diastolic volume was larger for the puppies (77 per cent) than for the full-grown dogs (60 per cent). In relation to their body weight the stroke volume for the full-grown animals was 60 per cent lower than that for the puppies. The pulmonary circulation time increased with age. It was shorter in puppies than in full-grown dogs, both absolutely and in terms of the number of cardiac cycles which it occupied. The prolongation of the pulmonary circulation time with advancing age, when expressed in terms of the number of heart cycles which it occupies, may be mainly due to decrease of the stroke volume in relation to the body weight.

Cineangiocardiography is a useful method for studying cardiac dynamics. As shown in a previous paper (Gribbe et al. 1959) it is possible to calculate the stroke and the residual blood volumes of the left ventricle, the cardiac output and the pulmonary circulation time from angiocardiograms, recorded at a high rate during the passage of blood containing radiopaque material through the heart cavities. In the present paper cineangiocardiographic findings in young and full-grown animals are compared.

Methods

We employed as our experimental animals 6 puppies, aged between 63 and 91 days and weighing 2.3—4.6 kg and 20 full-grown dogs weighing 7—19.3 kg

Table I Duration of various phases of the mechanical heart cycle in puppies and full-grown dogs

	Cycle length (msec)	Isometric contraction	
		msec	% of cycle
2—3-month puppies (9 experim.)	333 ± 13	48 ± 3	14.5 ± 0.7
Full-grown dogs (13 experim.)	420 ± 23	60 ± 3	14.6 ± 0.8

The animals were anesthetized with an intraperitoneal injection of Nembutal. The dose varied from 30 to 40 mg per kg of body weight.

A Cournand catheter or a polyethylene tube was introduced through the external jugular vein into the right heart. The injection of contrast medium (Urografin 76 %, Schering-Leiras, 1.4 ml per kg of body weight) was performed with a Gidlund automatic pressure syringe into the right atrium. The angiocardiograms were taken with a 5-inch Philips image intensifier and an Arriflex 35-mm camera employing an exposure rate of 48 pictures per second. The right oblique anterior projection was used. Electrocardiograms were taken simultaneously employing synchronous picture marking.

The diameters of the left atrial and of the left ventricular cavities could be measured frame after frame from the levogram and their volumes could be calculated by the formula given by Gribbe et al. (1959).

Results

The heart rate was slightly higher in the puppies (184/min.) than in the full-grown dogs (169/min.).

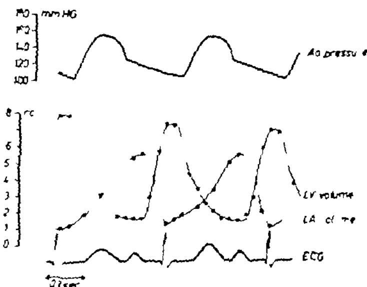


Fig. 1. Volume curves of the left heart of a puppy. Note the large stroke volume of the left ventricle in relation to the end-diastolic volume.

Means ± errors of the means

Ejection		Isometric relaxation		Filling	
msec	% of cycle	msec	% of cycle	msec	% of cycle
127 ± 7	38.4 ± 2.1	76 ± 5	23.0 ± 1.6	82 ± 9	24.1 ± 1.7
128 ± 12	30.6 ± 2.5	90 ± 5	22.3 ± 1.3	142 ± 18	32.5 ± 2.5

The various phases of the mechanical cardiac cycle can be calculated with sufficient accuracy by studying the films. The phases of the heart cycle are presented in Table I. The greatest difference is observed in the filling phase which is both absolutely and relatively longer in the full-grown dogs than in the puppies: 1.75 times as long in seconds and 1.45 times as long when computed as a fraction of the cardiac cycle. The isometric contraction and relaxation phases expressed in milliseconds were somewhat longer in the full-grown than in the puppies, but the ejection phase was of the same length in both groups.

Left ventricular volume curves for the two dog groups are shown in Fig. 1 and 2. The curves have been drawn in the correct time relationship to the simultaneously recorded electrocardiograms.

The results of the volume measurements are shown in Table II. The relative *end-diastolic volume* (in per mille of body weight) was slightly but not significantly larger in the puppies than in the full-grown dogs.

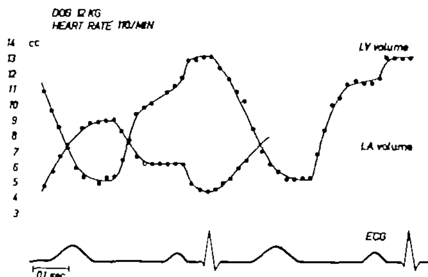


Fig. 2. Volume curves of the left heart of a full-grown dog. Note the length of the filling phase of the left ventricle.

Table II Results of volume calculations of the left ventricle in various animal groups

The numbers in parentheses give the ranges

Animal group	End-diastolic volume		Residual blood volume (ml)	Stroke volume			Heart rate	Minute volume ml/kg b.w
	ml	‰ of b.w		ml	‰ of b.w.	% of end-diastolic volume		
2–3-month-old puppies	4.9 (3.1–7.4)	1.59 ± 0.15	1.1 (0.6–1.6)	3.8 (2.3–5.8)	1.23 ± 0.10	76.7 ± 1.0	181 ± 7	223 (152–315)
Full-grown dogs	12.9 (3.9–23.5)	1.25 ± 0.07	5.1 (3.1–10.0)	7.8 (3.9–15.5)	0.73 ± 0.04	60.4 ± 0.7	169 ± 5	124 (79–151)

The stroke volume relative to the body weight was 1.23 per mille of body weight for the puppies. A comparison of the stroke volume of the puppies and full-grown dogs (*0.73 per mille*) revealed a significant decrease (difference 0.48 ± 0.11 per mille) with increasing age.

The stroke volume expressed as a percentage of the end-diastolic volume decreased clearly with age. The difference in mean stroke volume is significant between the puppies and full-grown dogs. The stroke volume was 77 per cent of the end-diastolic volume in the puppies and 60 per cent in the full-grown dogs the difference is 17 ± 1 per cent.

This means that *the residual blood volume* expressed as a percentage of the end-diastolic volume was smaller in the younger animals than in the full-grown ones.

As seen from Table II *the minute volume* per kg of body weight was larger in the puppies than in the full-grown dogs, the mean value being 225 ml for the former group and 124 ml for the latter group.

The pulmonary circulation times expressed as the number of heart cycles are shown in Table III. The mode was obtained for two-thirds to three-fourths of the animals in the various groups.

The deviations from the mode were not great. The number of heart beats needed to force the blood from the proximal part of the pulmonary artery to the left atrium increased with the age of the animals.

A comparison of the angiocardiographic films, electrocardiograms and pressure curves showed that extrasystoles, which are frequently seen in selective angiocardiography had no, or only a slight, ability to transport the blood into the pulmonary vessels. On the other hand, determinations of the volume of the left ventricle revealed that the first beat after the extrasystole started from a greater end-diastolic volume and resulted in a greater stroke volume than the beats before the extrasystole.

Table III. Pulmonary circulation times in seconds and in numbers of heart cycles

Extrasystoles are indicated by

Animal group	Sec.	Number of cycles	Range
2—3-month-old puppies	1.0	2 +	2 – (2 +)
Full-grown dogs	1.7	4	4 – 5

The occurrence of extrasystoles did not, however lead to any uncertainty in the results of the experiments. In the puppies the pulmonary circulation time never exceeded two heart cycles and an extrasystole, but in the full-grown dogs the circulation time was 4—5 cycles.

Discussion

From the results presented it appears that the relative stroke volume (as a per centage of the end-diastolic volume) decreases, and the residual blood volume increases with age. The stroke volume is a most important indicator of the heart work and several investigators have studied the stroke volume in adults and children. W th the introduction of cineangiocardiography it was possible for the first time to determine simultaneously the stroke volume and the amount of residual blood in the left ventricle. The method was used for studying the volume of the left ventricle by RUSHMER and THAL in 1951 and by CHAPMAN and BAKER in 1958. The former did not calculate the volume as such, but used the ventricular area in a single plane as an index to the volume. The latter presented both the method and the results from two cases, dog and man. The results, however were very different. The residual blood volume was determined to 15 per cent in the man and 65 per cent in the dog

Determinations of stroke and residual blood volumes in full-grown dogs have previously been presented by GAMBLE et al. Good correlation between the Fick direct method for determining the minute volume was established.

Until now no results have been published on comparative studies of the residual blood volume between fullgrown and young subjects.

The striking difference demonstrated between the relationship stroke volume/ residual blood volume in growing and full-grown subjects is apparent.

The young heart cannot meet increased requirements except by increasing the heart rate (REIN and SCHNEIDER 1956)

The pulmonary circulation time has been discussed only in a few reports. In 1927 BLUMGART and WEISS employed Radium C to determine the pulmonary circulation time in man. They found the average pulmonary circulation time in adults t be 6.5 sec or 7—8 heart beats. In 1955 NEAI et al. used fluorodensography for this purpose and observed that the circulation time from the right

ventricle to the left ventricle takes 4 sec or 3 1/2 heart beats on an average. In 1954 NORDENSTRÖM measured the circulation time from the pulmonary artery to the left atrium in full-grown dogs by densitometry and found it to vary from 2.1 to 3.5 sec. It can be calculated from his figures that this corresponds approximately to 6—9 heart beats. It is possible that the first signs of contrast medium are seen earlier in the left atrium in angiocardiographic films than they are observed by the above method, and hence this may be the reason why we found a smaller number of heart beats in our dog series. In connection with roentgen-cinematographic angiocardiography on man JANKER and HALLERBACH (1951) observed a pulmonary circulation time of 3.29 sec which corresponds to four heart cycles.

We were unable to find any information from the literature about comparative studies of the pulmonary circulation time in different age-groups. Our experiments showed a prolongation of the pulmonary circulation time with advancing age and body size, not only in time units but also when it was expressed as the number of cardiac cycles. This prolongation was probably associated with the change with age in the relative stroke volume. The mean stroke volume was 0.75 per mille of the body weight for the full-grown animals, which was 40 per cent lower than the mean stroke volume, 1.23 per mille, for the puppies.

Other factors e. g. pulmonary vascular resistance, pulmonary blood pressure, short circuits, blood volume in the pulmonary vessels and the relative size of the heart and lungs at various ages may exert an influence on the pulmonary circulation time. This study could not take into account the possible importance of these factors.

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Effects of Intravenous Infusions of Adrenaline and Noradrenaline on Certain Psychological and Physiological Functions

by

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Abstract

FRANKENHAEUSER, M., G. JÄRPE and G. MATELL. *Effects of intravenous infusions of adrenaline and noradrenaline on certain psychological and physiological functions.* Acta physiol. scand. 1961. *51* 175—186. — Effects of intravenously infused l-adrenaline (average dose 14.9 µg/min, average infusion time 34.7 min) and l-noradrenaline (average dose 16.2 µg/min, average infusion time 36.8 min) were examined by objective methods in ten subjects. The psychological tasks were chosen so as to represent different functional areas. Heart rate and arterial blood pressure were continuously recorded, and excretion of urinary catechols before, during and after infusions was determined. Both drugs produced subjective symptoms (mental and physiological) and these were more pronounced and more frequent for adrenaline. Marked changes in heart rate and arterial pressure were recorded during the infusions. The stress involved in performing the psychological tasks also caused a pronounced increase in heart rate, arterial pressure and the urinary excretion of catechols. Performance did not differ significantly from that during the infusion of Ringer solution. These results were tentatively interpreted to indicate that an increased alertness accompanying the emotional changes produced by the drugs may act to counterbalance other unfavourable effects on performance.

The main aim of the present experiments was to study effects of intravenously infused adrenaline and noradrenaline on certain psychological functions as measured by objective techniques.

Though the general effects of infused adrenaline on physiological processes and emotional state are well known, quantitative data as to effects on perform-

ance are scarce. JERSILD and THOMAS (1931) examined the effects of subcutaneous injections of adrenalin hydrochloride (0.4—0.9 mg) and found a slight improvement in motor tasks (tapping and strength of grip) but no change in other psychological functions. LANDIS (1935) investigated the influence of subcutaneous injections of adrenalin (1 mg adrenalin hydrochloride, Parke, Davis & Co) on complex muscular activity (dart throwing) expecting to find an impairment in speed and accuracy but no change could be demonstrated. BASOWITZ et al. (1956) found that motor performance was significantly impaired during prolonged intravenous adrenaline infusion at a low dose level (5 μg/kg body wt/hr) whereas performance in other types of tasks was not affected.

Psychological effects of noradrenaline do not seem to have been studied by quantitative methods. However the general opinion, based on observations and introspective accounts of subjects, appears to be that noradrenaline does not cause marked psychological changes (*e.g.* SWAN 1952)

Data provided by studies of performance in other types of stress-situations (failure stress, task-induced stress etc.) do not present a clear picture. The problem is highly complex and it is evident that stress may both aid and interfere with performance depending upon a number of factors, such as the degree and nature of the stress, the type of performance as well as various personality traits of the subject (LAZARUS, DEESE and OSLER 1952, STENNBERG 1959)

Some of the mental symptoms commonly occurring during infusion of adrenaline, such as increased mental alertness, may be expected to improve performance, whereas others, such as severe anxiety may be accompanied by a deterioration in performance Hence it did not seem possible to make predictions as to specific effects on performance Neither could such predictions be made in respect of noradrenaline.

On the basis of these considerations the most adequate mode of approach seemed a relatively broad, explorative study which would provide more information as to the effects of these drugs on various aspects of behaviour A battery of tests was put together which included samples from several areas of psychological functioning psychomotor performance, power of concentration, memory span, word fluency time estimation and a colour word test.

In addition, heart rate and arterial blood pressure were recorded and urinary excretion of adrenaline and noradrenaline was examined.

By examining each subject during infusion of adrenaline noradrenaline and Ringer's solution (control condition) it should be possible to determine if certain aspects of behaviour are particularly sensitive to the drugs. Furthermore the concomitant physiological recordings should make it possible to relate physiological and psychological data to each other Hence the information gained by these experiments should provide the necessary basis for further investigations on more specific problems.

Methods

Subjects

Six male university students participated in the experiments. Their ages ranged between 19 and 31 years (average 23.5 years) and body weights and heights between 63 and 71 kg (average 67.7 kg) and 177—190 cm (average 182.2 cm) respectively.

Infusions

Catecholamines and placebo solutions were given as continuous i. v. drip during periods ranging between 33 and 37 min. The catecholamines were given in solutions

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Urinary catechols. Immediately after voiding, the urinary volume was measured and pH adjusted to 3.5 with N HCl. Specimens were then stored at + 4° C until analysis, which was performed using a fluorometric technique (EULER and LISHAJKO 1959).

Psychological variables

The test battery comprised 7 tasks, which were always given in the order listed below.

100-7 test. The task was to count backwards from 100 by subtracting seven each time. The score was the time taken to complete the count.

Tapping speed. The task was to tap as rapidly as possible with the right hand index finger on a key connected to an electric counter. The score was the number of taps in 30 sec.

Time estimation. Present- and past time estimates of 4 time periods between 9 and 37 sec were obtained using the method described by FRANKENHAEUSER (1959 p. 38 ff). Scores for present, past and retained time were calculated (cf. FRANKENHAEUSER 1959 p. 40).

Colour-word test. One part (card C) of the Stroop test (THURSTONE and MELLINGER 1953) was used. This consists of 100 colour words (blue, red, yellow, green) printed in random order on a gray sheet. The combination of words and colours is incongruent, so that the word yellow may be coloured red etc. The task is to name the *colour* of the print, ignoring the word, as quickly as possible. The score was the time taken to complete the task.

¹ Nor Exadrin conc. ASTRA, Södertälje, Sweden.

Word fluency. The task was to write down as many words as possible that start with *s* and end with *a*. The time allowed was 5 minutes and the score was the number of words correct.

Mirror drawing. The task was to move a stylus along a track cut out in a brass plate so as to form a 5-pointed star. This track was visible only in a mirror. When the stylus touched the margins of the track a circuit was completed (for details see FRANKENHAEUSER, HEMMER and GRAFF-LONNEVIG 1960). A time score was calculated as the mean of the time taken to complete 4 consecutive trials and an error score as the mean time of contact between stylus and margins.

Memory span. The task was to repeat digits read by the examiner a) forwards, and b) backwards. The scores were the maximum number of digits correctly repeated.

Peg-washer test (DUREMAN and SÄLDE 1959, p. 17). The task was to move nobbed pegs from one side to another of a drilled board. A washer had to be fitted on each peg before it was replaced on the board. The time score was the time taken to move 50 pegs, and the error score the number of washers and pegs dropped.

Experimental design

Each subject came to three experimental sessions, in which infusions of adrenaline noradrenaline and Ringer's solution, respectively were given. The procedure was exactly the same each time and the subject did not know which infusion was given. Each subject had his three sessions at the same time of the day and the intervals between sessions ranged between 5 and 8 days.

In order to counterbalance effects of practice on performance and of general adjustment to the experimental situation, the three conditions were rotated as follows.

Subject	Session I	Session II	Session III
1	Adrenaline	Noradrenaline	Ringer solution
2	Noradrenaline	Ringer solution	Adrenaline
3	Ringer solution	Adrenaline	Noradrenaline
etc			

This design was repeated for the three remaining subjects.

Under each condition the procedure was as follows. The subject emptied his bladder. Preparations were made for intravenous infusion and recording of blood pressure and heart rate. The first sample of urine was obtained after about 1 hour, when all preparations for the experiment were completed. The subject was comfortably seated and his ability to move his arms and hands freely and without pain was checked. He was told that the infusion would begin shortly and asked to report any symptoms that occurred. (Measuring units and infusion aggregate were placed behind the subject so as to prevent him from following the procedure. Some minutes later the infusion was started without further announcement to the subject. A few minutes after the infusion had begun the psychological testing was started. It was completed in about 30 minutes upon which the infusion was immediately interrupted and a second sample of urine was collected. This sample represented an average span of 40 minutes. The subject was told to relax while the recordings of heart rate and blood pressure were continued for about 25 minutes, when a third sample of urine was collected. Finally the subject was questioned about his symptoms and experiences before, during and after the infusion. The questioning followed a special check-list of symptoms and was thus invariably the same for all subjects under all conditions.

Table I. Subjective symptoms during adrenaline and noradrenaline infusions

	Adrenaline Infusion Subject							Noradrenaline infusion Subject							Ringer Infusion Subject						
	1	2	3	4	5	6	f	1	2	3	4	5	6	f	1	2	3	4	5	6	f
Mental symptoms																					
Restlessness	+	+	+	+	+		5	+			+	+		3							0
Agitation	+	+			+		3					+		1							0
Anxiety	+						1							0							0
Tenseness	+	+		+	+		4	+			+	+		3							0
Discomfort	+	+		+			3	+			+			2							0
Physiological symptoms																					
Palpitation	+	+	+	+	+	+	6		+	+	+	+	+	5					+		1
Tremor	+	+	+	+	+	+	6							0							0
Perspiration	+			+			2				+			1							0
Numbness	+			+	+		3				+			1							0
Dyspnoea	+			+	+		3				+	+		2							0
Pounding in head, abdomen and thorax	+	+		+			3		+		+			2							0

Results

Subjective symptoms

The subjective symptoms experienced during adrenaline infusions had the same general characteristics as those described in previous investigations on healthy human subjects (e.g. EULER, LUFT and SUNDIN 1954; DURESMAN and SCHOLANDER 1956). Noradrenaline produced some effects similar to those of adrenaline, but these symptoms were on the whole less pronounced and less frequent. Symptoms mentioned by the subjects, either spontaneously or in response to the check-list questions, are listed in Table I. The table shows that there are large differences between subjects in frequency of symptoms. Subject 1, for example, reported 5 mental and 6 physiological symptoms, whereas subject 6 had no mental and only 2 physiological symptoms. The only symptom reported during infusions of Ringer's solution was one case of palpitation.

Effects of infusions on psychological variables

Though some subjects felt strongly affected by adrenaline, all were able to cooperate satisfactorily in the psychological testing. The mean scores are summarized in Table II. For those tests in which scores are obtained in physical time, and where consequently the unit of measurement is constant, performance

Table II. Means and S.E. of scores in performance tests during infusions of adrenaline, noradrenaline and Ringer's solution. Figures indicate time in sec except for Tapping, Word-fluency, Memory span and Peg-washer error where frequency is indicated

Variable	Scores during infusion of		
	Adrenaline	Noradrenaline	Ringer solution
100—7 test	15.8 ± 2.37	21.5 ± 6.15	20.0 ± 3.97
Tapping speed	*221.5 ± 8.71*	*228.4 ± 11.4*	*223.8 ± 5.97*
Colour word test	79.0 ± 6.42	80.7 ± 7.86	86.2 ± 9.63
Word-fluency	28.0 ± 3.47	31.0 ± 3.20	31.8 ± 4.41
Mirror drawing, time	15.9 ± 1.62	14.7 ± 1.02	11.3 ± 1.56
Mirror drawing, error	3.9 ± 0.33	3.5 ± 0.45	3.1 ± 0.08
Memory span			
digits forwards	7.8 ± 0.32	7.5 ± 0.50	7.5 ± 0.43
digits backwards	6.2 ± 0.70	6.0 ± 0.58	6.2 ± 0.47
Peg-washer test, time	181.4 ± 18.40	175.3 ± 10.90	170.3 ± 15.10
Peg-washer test, error	2.4 ± 0.96	1.2 ± 0.40	1.2 ± 0.31

under adrenaline and noradrenaline conditions has also been expressed as per cent of performance during Ringer's infusion (Table III). The most striking result is that the differences in performance under the various conditions were on the whole small, in many cases even negligible. None of the differences reached statistical significance.

If the results are taken at their face value, they seem to indicate that the effects of adrenaline are stronger than those of noradrenaline and that adrenaline may improve performance in tests where speed plays a decisive role, except in the case of psychomotor ability where performance seems impaired. However, the differences obtained may be due to chance and a much larger material would be required to confirm these tentative interpretations.

In time estimation a small but consistent difference between adrenaline and control scores was obtained, present and past time estimates both being

Table III. Performance (time scores) during infusions of adrenaline and noradrenaline in per cent of performance during infusion of Ringer's solution

Variable	Adrenaline infusion	Noradrenaline infusion
100—7 test	79	108
Colour-word test	97	92
Mirror drawing, time	111	103
Mirror drawing, error	126	106
Peg-washer test, time	108	103

Table IV Means and S. E. of heart rate, systolic and diastolic blood pressure before, during and after infusions of adrenaline and noradrenaline

Variable	Adrenaline infusion			Noradrenaline infusion		
	Before	During	After	Before	During	After
Heart rate	78 ± 6	99 ± 8	91 ± 6	77 ± 5	63 ± 4	76 ± 8
Systolic blood pressure	143 ± 5	190 ± 7	136 ± 7	126 ± 4	166 ± 8	122 ± 6
Diastolic blood pressure	83 ± 3	88 ± 5	80 ± 7	77 ± 4	96 ± 7	73 ± 3

larger during adrenaline. This effect is analogous to that produced by metamphetamine (FRANKENHAEUSER 1959). The influence of adrenaline on time estimation will be examined further in a separate series of experiments.

Effects of infusions on physiological variables

The mean heart rate, systolic and diastolic blood pressure of each subject before, during and after each infusion was calculated. The means of the individual means thus obtained are shown in Table IV. The changes seen are in general agreement with those obtained by previous investigators (e.g. EULER et al. 1954, GOLDENBERG et al. 1948). The only unusual feature is the rise (instead of expected fall) in diastolic pressure under adrenaline. A possible explanation is that the rise was caused by the combined effects of the infused adrenaline and the stress produced by the psychological tests (cf. p. 8).

The results of the analyses of urinary catechols are shown in Table V. It should be noted that the adrenaline values *before* infusions are higher than those obtained by previous investigators (EULER and LUFT 1951, EULER et al. 1954). This may be a consequence of the stress induced by the catheterization of blood vessels and it may also be an effect of anticipation of the experiment of follow-

Table V Means and S. E. of catecholamine excretion in urine before, during and after infusions of adrenaline and noradrenaline

Variable	Adrenaline infusion			Noradrenaline infusion		
	Before	During	After	Before	During	After
Excreted adrenaline mμg/min	15.3±2.7	598.2±30.1	236.8±32.6	20.2±4.5	52.0±6.3	29.5±8.5
Excreted noradrenaline mμg/min	21.6±5.4	0.9±1.0	14.9±3.9	28.4±4.3	478.4±68.2	92.6±23.9

Table II. Means and S. E. of heart rate, diastolic and systolic blood pressure and the excretion of catecholamines in urine before, during and after infusions of Ringer's solution with and without concomitant psychological testing

Variable	Infusion of Ringer sol. plus psychol. testing			Infusion of Ringer sol. only		
	Before	During	After	Before	During	After
Heart Rat	73 ±5	78 ±6	70 ±5	72 ±4	68 ±4	69 ±3
Systolic blood pressure	124 ±7	132 ±9	125 ±6	116 ±4	124 ±4	119 ±4
Diastolic blood pressure	66 ±4	73 ±4	67 ±4	81 ±2	83 ±4	83 ±2
Excreted adr in mμg min	16.6±3.1	35.4±7.0	13.5±4.5	14 0±2.4	15.3±2.7	12.2±2 6
Excreted noradr in mμg min	21.2±4 1	31.2±3.7	20.3±5.3	18.7±2.5	16.2±0 6	15.2±2.3

A second striking feature is the large amount of adrenaline excreted during the infusion period. The per cent output of infused adrenaline averages 5.01 (range 4 17—5.61) which is considerably more than that found in earlier investigations (EULER et al. 1954) In respect of noradrenaline the corresponding values are 3.22 (range 2 38—4 17) which is consistent with earlier findings (EULER and LUFT 1951)

Effects of psychological tests on physiological variables

During the infusions of Ringer's solution an increase in heart rate blood pressure and excreted catechols was noted In order to find out if these changes were caused by the infusion *per se* or by the psychological tests, or both, a new series of experiments with infusion of Ringer's solution only was carried out with the same six subjects. The procedure was exactly the same as before except that the psychological tests were substituted by the reading of various magazines with a neutral and unengaging content. Table VI shows the results from both series with Ringer's solution.

In the second series (without testing) the differences between measures obtained before and during infusion are either much less pronounced or abolished. Hence it may be concluded that the changes in the first series were primarily caused by the psychological tests and not by the infusion.

Another striking example of physiological reactions to the stress induced by psychological tests was seen in the continuous recordings of arterial pressure and heart rate as illustrated in Fig 1 Performance — particularly in stressful tasks, such as the Colour-word test and Mirror drawing — is accompanied by distinct rises in systolic blood pressure The figure also shows the rise in blood pressure caused by a test (Peg-washer) given before and after the infusions.

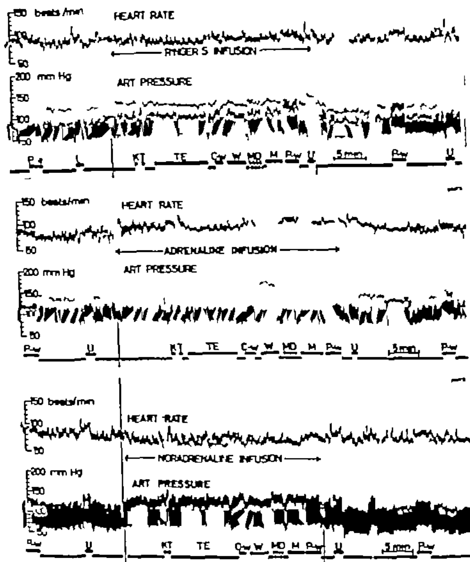


Fig. 1. Recording of heart rate and arterial blood pressure before, during and after infusions of adrenaline, noradrenaline and Ringer solution. Time for psychological tests is marked in the bottom tracing. P-w = Peg-washer test. U = Urine sample; the rise in blood pressure is in this case caused by the subject standing up. K = 100-7. T = Tapping speed. TE = Time estimation. C-w = Colour-word test. W = Word fluency. MD = Mirror drawing. M = Memory span.

Discussion

The present experiments have provided some quantitative information as to the ability to carry out various kinds of activities during infusions of adrenaline and noradrenaline in relatively large doses. Though inter-individual reactions to the drugs were large, especially in respect of adrenaline, all subjects were able to complete the series of tests and no signs of an approaching break-down of performance were noticed.

The tremor during the adrenaline infusions made the psychomotor tasks more difficult to perform. However, most subjects seemed to compensate to some extent for this handicap by an intense effort to concentrate on the tasks. It thus appeared as if the unfavourable effects of decreased hand steadiness were counterbalanced by the favourable effects of increased alertness, thus enabling the subjects to carry out the tasks without appreciable impairment. However, it seems evident that performance changes should depend upon the doses employed, and that with increasing doses the negative effects would sooner or later dominate. It also appears reasonable to assume that, on the other hand, with smaller doses a stage could be found where only favourable effects on performance ensue.

In future experiments specific hypotheses may be based on the observations made in the present series, that there was a tendency for psychomotor performance to deteriorate and for other types of performance, requiring primarily speed, to improve during adrenaline infusion.

It should be kept in mind that the testing, for various technical reasons, was not started until a few minutes after the infusion had begun, i.e. not until the initial subjective symptoms, which are usually the strongest, had more or less subsided. It is possible that a sudden and temporary change in performance took place in this initial phase. During the subsequent period, when the tests were carried out, the subjective state as well as heart rate and blood pressure remained fairly stable.

The effects observed of psychological factors on physiological functions are of particular interest. It was clearly shown that the performance of the psychological tasks was accompanied by an increase in heart rate, arterial pressure and urinary excretion of catechols. Furthermore the stress involved in anticipation of the test period and/or the concomitant catheterization of blood vessels produced an adrenaline excretion considerably above normal.

A striking feature of the adrenaline experiments was the high percentage adrenaline recovered in the urine. One possible explanation may be that the diuresis was large in most cases because of extra water intake to secure prompt micturition. This interpretation is supported by a statistically significant positive correlation between diuresis and amount of catechols in the urine.

There has been considerable debate as to the question whether the emotional experiences produced by adrenaline injections are "real" emotions or whether

they are more adequately described as "cold" or "as-if" emotions, i. e. the subject feeling *as if* he were afraid, anxious etc. though he is not. The various observations have been critically reviewed by LANDIS and HUNT (1932) who conclude that injections of adrenaline but rarely produce genuine emotions. In the present experiments the emotional reactions to adrenaline were definitely of the "as-if" type. Furthermore the subjects appeared very reluctant to admit any severe emotional changes and seemed to attempt to ignore their symptoms as far as possible. It is evident that a successful penetration of these problems requires more refined techniques and at present various methods (ratings, interviews, projective techniques) for measurements of emotional experiences during adrenaline and noradrenaline infusions are being tried out.

Possible selective effects of adrenaline versus noradrenaline infusions on emotional changes are of particular interest in view of the relationships observed between certain specific psychological and physiological response patterns, fear being associated with "epinephrine-like" and anger with "norepinephrine-like" cardiovascular reactions (cf FUNKENSTEIN 1956). More direct evidence for a selective function of adrenaline and noradrenaline in emotion has been provided by studies where excretion of urinary catechols during stress has been compared with the nature of emotional responses. Such experiments indicate that anxiety versus aggression, is related to a relatively larger increase in adrenaline versus noradrenaline. (ELMADJIAN, HOPE and LAMSON 1957 SILVERMAN, COHEN and ZUIDEMA 1957) In the present experiment qualitative differences in emotional reactions to the two drugs were not observed. It is, however, possible that differences may be revealed by special techniques, such as suggested above.

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On the Nervous Regulation of the Biliary System in the Cat

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Abstract

PALLIN B and S. SKOGLUND *On the nervous regulation of the biliary system in the cat* Acta physiol scand. 1961 *51* 187—192. — The nervous regulation of the biliary system was investigated on cats. Stimulation of the vagal nerves was shown to increase the pressure in the gallbladder and the hepatic duct, whil stimulation of the splanchnic nerves was found to inhibit the action of the vagal nerves. From these experiments is concluded that the vagal nerves exert 'secretory' action on the biliary system, while the splanchni nerves inhibit the outflow of bile. The mechanisms underlying the interaction of these facilatatory and inhibitory actions are briefly discussed.

It is well known that the biliary system recieves its nerve supply from the right and left vagus nerves and th coeliac ganglia. Whether the phrenic nerves contribute any fibres to this innervation is not known. However t has been shown by ALEXANDER (1940) that these latter nerves join the sympathetic and para sympathetic ams which enter the liver The nerves directed to the biliary system ccompany the hepatic artery and the portal vein and according to ALEXANDER (1940) the fferent innervation of the blood vessels is sympathetic and the innervation of the bile ducts both sympathetic nd parasympathetic. The function of these nerves have been the subject of some investigations (TANTURI and IVY 1938, JOHNSON and BOYDEN 1943, POLLIEUX et al 1952) In these works t was shown that the vagus nerves are 'secretory that is, when stimulated bile is released from the biliary system. If the bile formation in the li er is under direct nervous control is uncertain, but TANTURI and

Table I

Cat	IP	Right vagus stim.	Left vagus stim.
1	0	0.8	0.1
2	0	1.3	0.2
3	0	0.7	0.4
4	0	0.7	0.1
5	0	0.4	0.3
6 M	0	1.1	0.0
7 M	0	1.4	0.3
average		~0.9	~0.2

Table I showing the effect of vagal stimulation. The *splanchnic outflow intact. Initial pressure (IP) set at zero.* Pressure variations measured by strain gauge manometer with polyethylene catheter in the gallbladder. Figures demonstrate pressure alterations in cm H_2O.

Ivy (1938) suggested that there might be some direct nervous influence on the secretion of bile from the liver on dogs. Stimulation of the sympathetic nerves of the biliary system has been shown to exert an inhibitory action on the output of bile (Johnson and Boyden 1943) and to inhibit the output of bile from the liver (Tanturi and Ivy 1938).

The literature gives no further information about the nervous regulation of the biliary system and furthermore most of the knowledge has been gained from inspection of the volume variation of the gallbladder which must be considered a rough method. It was therefore considered worth while reinvestigating the control exerted by different nerves acting on the biliary system recording the effects as pressure variations of closed systems.

Methods

Fifty-two cats were used. They were anesthetized with Nembutal (Abbot 40 mg/kg body weight intraperitoneally). A tracheal cannula was routinely inserted. Thoracotomy was performed on the right side. The tracheal cannula was connected with a pump for artificial respiration. The sixth to the tenth rib was resected to the sternal bone and the *inferior lobe of the right lung was extirpated.* The vagus nerves were dissected free just above the diaphragm. The abdomen was opened in the midline. This approach was used in all experiments.

In one series of experiments a polyethylene catheter was inserted into the gallbladder *according to the technique demonstrated by Ivy and Jansen (1959)* and connected to a sensitive inductive strain-gauge manometer. In some experiments indicated by M a manometer was used for pressure measurements according to the method by Ivy and Jansen (1959). In all the experiments the initial pressure (IP in the tables) set to zero was decided three times with an interval of three minutes. The cystic duct was dissected

Table II

		A.			B.		
Cat	IP	Spl. n. intact	Spl. n. cut	Diff.	Spl. n. intact	Spl. n. cut	Diff.
1	0	0.8	1.7	0.9	0.1	0.3	0.2
2	0	1.3	1.5	0.2	0.2	0.6	0.4
3	0	0.7	1.7	1.0	0.4	0.7	0.3
4	0	0.7	1.6	0.9	0.1	0.4	0.3
5	0	0.4	1.5	1.1	0.3	0.4	0.1
6 M	0	1.1	2.2	1.1	0.0	0.6	0.6
7 M	0	1.4	1.8	0.4	0.3	0.6	0.3
average				=0.8			=0.3

Table II showing effect of vagal stimulation (A, right vagus and B, left vagus) before and after sectioning of the greater lesser and least splanchnic nerves. Initial pressure set at zero. Pressure variations measured by straingauge manometer with polyethylene catheter in the gallbladder Figures demonstrate pressure alteration in cm H_2O.

free from vessels and ligated. The dissection was made very carefully not to exert pressure on to the gallbladder during the operation which causes bile outflow The thoracic cavity was filled with paraffin oil at 37° C and kept at that temperature throughout the experiment.

In another series of experiments the same operative approach as in the first series was used but the polyethylene catheter was now inserted into the hepatic duct, which was dissected free from the vessels and the nerves. The pressure was recorded in the same way as described above When using the I—y and JANECEK technique, the trochar was connected to polyethylene catheter which was inserted in the hepatic duct.

In both series of experiments the dissected nerves were stimulated electrically by supramaximal shocks at frequencies between 5 and 36 sec. By recording the action potentials it was always checked that the nerves were supramaximally stimulated.

Results

In the first series of experiments, in which the pressure was measured in the gallbladder after ligation of the cystic duct, stimulation of the peripheral ends of vagal nerves in 7 animals showed, that the right one had a more potent effect than the left (see Table I) When the right vagus was stimulated, the pressure of the gallbladder increased 0 9 cm H O When the left one was stimulated the rise was only 0.2 cm H O

When these effects had been established the greater lesser and least splanchnic nerves were cut before they reached the coeliac ganglia (7 animals) When now stimulating the right and left vagus the pressure in the gallbladder increased 0.8 cm and 0.3 cm H_2O respectively (see Table II) From this it can be concluded that the sympathetic fibres have some inhibitory action on the release of bile from the gallbladder

Table III

	A.				B.		
Cat	IP	Symp. n. intact	Symp. n. cut	Diff	Symp n. intact	Symp. cut	Diff.
1	0	0.5	2.1	1.6	0.0	0.5	0 5
2	0	0 8	2.2	1 4	0.2	0.6	0 4
3	0	0.7	1 7	1.0	0.1	0.7	0 6
4	0	0.9	1.9	1.0	0.3	0.9	0 6
5	0	1.0	2.6	1 6	0 1	0.6	0 5
average				–1.3			–0 5

T ble III showing the effect of vag l stimulation (A, right vagus nd B left vagus) before and after sectioning all branches contributing t the splanchnic nerves from th 4 to Ll and removing the sympathetic trunks from the diaphragm to the ganglion impar on both sides. Initial pressure set at zero. Pressure ariations measured by a train-gauge manometer with polyethylene catheter in the gallbladder Figures demonstrat pressure changes in cm H_2O

In another series of experiments all branches contributing to the splanchnic nerves from Th 4 to L 1 were cut in 5 animals, and the whole sympathetic trunks from the diaphragm to the ganglion impar was removed on both sides. This operation wa performed after having decided the effects of vagal stimula tion in the intact animal It was now found (see Table III) that the differences in effect on the pressure produced by vagal stimulation in the gallbladder was very small compared with those in which the splanchnic nerves had been cut Consequently the influence of direct sympathetic fibres on the gallbladder must be small.

In the experiments reported hitherto the effects of the sympathetic outflow from both sides have been considered. To determine any possible differences between the rigl t and left side the splanchnic nerves on the right side were cut in 5 animal after having decided the effect of vagal stimulation in tl e i tact animal. It wa then found (see Table IV) that most of the inhibition exerted by the splanchnic nerves emanated from the right nerves.

In 6 an mals only the greater planchnic nerve was cut and the rigl t and left vagal nerves stimulated peripherally The pressure rise now obtained wa nearly as great as when all splanchnic nerves had been cut From these experiments the onclusion can be drawn that the inhibition of the galll ladder from the lesser and lea t spla chnic nerves is less potent than that from tl e greater one.

In 4 animals the right agus nerve wa stimulated simultaneously with the right greater splanchnic nerve The pressure in the gallbladder as then found to increase very much less than on vagal timulation alone This shows that the sympathetic influence on the gallbladder is inhibitory on the action of the parasympathetic

Table IV

A.					B.		
Cat	IP	Spl. dx. intact	Spl. dx. cut	Diff.	Spl. dx. intact	Spl. dx. cut	Diff.
1	0	0.9	1.5	0.6	0.1	0.3	0.2
2	0	0.9	1.7	0.8	0.3	0.7	0.4
3	0	0.6	1.3	0.7	0.0	0.3	0.3
4	0	0.9	1.4	0.5	0.3	0.4	0.1
5	0	0.7	1.6	0.9	0.5	0.6	0.1
average				−0.7			−0.2

Table IV showing the effect of vagal stimulation (A, right vagus and B, left vagus) before and after sectioning of the splanchnic nerves on the right side. Initial pressure set zero. Pressure variations measured by strain-gauge manometer with polyethylene catheter in the gallbladder. Figures demonstrate pressure changes in cm H_2O.

TANTURI and IVY (1938) described a reflex in which stimulation of the central end of one vagus nerve with the other intact resulted in an increase of the flow from the liver. They could not produce this reflex in the cat. This experiment was here repeated while measuring the pressure in the gallbladder. In 4 animals the left vagus was cut and stimulated centrally. A pressure-rise of 0.3 cm H_2O was recorded in the gallbladder. In another series of 4 animals where a pressure rise was first established in the intact animal the right vagus nerve was cut and now stimulation at the central end of the left vagus nerve caused no pressure increase in the gallbladder.

In another type of experiments the catheter was inserted into the hepatic duct and the vagal nerves were stimulated before and after section of the sympathetic outflow to the liver. The aim of these experiments was to study the effects that could be induced on the release of bile.

The vagal nerves were stimulated in 7 animals as described in earlier experiments. A pressure increase of 0.7 cm and 0.2 cm H_2O respectively could be produced, which shows that the parasympathetic nerves in some way increase the intrahepatic pressure. The rise was obtained after stimulating a few minutes.

In 7 animals all splanchnic roots were cut before they reach the coeliac ganglia and when now stimulating the right and left vagus nerves peripherally the pressure-increase recorded in the hepatic duct was greater than before nerve-section. The splanchnic nerves therefore must have an inhibitory action on the effects that the vagal nerves exert on the liver.

In another 7 animals all splanchnic nerves were cut, the sympathetic trunks removed, and the phrenic nerves cut in addition. Stimulation of the right and left vagus nerves at their peripheral ends now resulted in a greater pressure-rise than with section of the splanchnic nerves alone. The phrenic nerves and the sympathetic trunks thus seem to have some inhibitory action on the pressure-increasing effect of the vagal nerves.

Discussion

The results presented show that the vagal nerves act as 'secretory' nerves, that is when stimulated they increase the pressure in the gallbladder. This indicates that these nerves have a tonic effect on the wall of the gallbladder. On the other hand the splanchnic nerves exert an inhibitory action on the tonus and decrease the effect induced by the vagal nerves.

The pressure-variations obtained on stimulation of the vagal nerves were small, but this was not unexpected because such small variations were also found by IVY and JANECEK (1959) when they stimulated the gallbladder-emptying mechanism by the use of cholecystokinin.

The results presented partly confirm earlier observations by TANTURI and IVY (1938) and JOHNSON and BOYDEN (1943). JOHNSON and BOYDEN did only determine the time necessary for gallbladder-evacuation by X-ray technique. They found by nerve-sections that the vagal nerves must have a 'secretory' action which is inhibited by the splanchnic nerves. In their experiments the sphincter of Oddi was included. In our experiments on the other hand, the cystic duct was ligated thus leaving out any effects on the sphincter of Oddi. The ligation of the cystic duct might destroy some nerve-fibres running to the gallbladder but as the major part accompanies the cystic artery which was dissected free, most of the innervation was probably intact.

TANTURI and IVY (1938) demonstrated a reflex action via the vagus on the release of bile from the liver in the dog. This was not investigated here but a reflex acting via the vagus on the gallbladder could be demonstrated.

In the experiments where the pressure was recorded it was found that stimulation of the vagal nerves increase the release of bile from the liver. TANTURI and IVY (1938) reported a direct effect of vagal impulses on bile-production on dogs. However they could not produce this effect in the cat. These effects might, however as suggested by TANTURI and IVY and MALLET-GUY et al. (1952) be induced by blood pressure or blood flow variations in the liver. The possible mechanisms behind these effects will be taken up in further work. The the interesting facilitating and inhibiting mechanisms acting on the gallbladder tone which seem to present an excellent experimental situation for the testing of humoral and nervous intergration.

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Noradrenaline Release from Isolated Nerve Granules

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Abstract

EULER, U. S. and F. LISHAJKO. *Noradrenaline release from isolated nerve granules.* Acta physiol. scand. 1961. *51.* 193—203. — Some properties of isolated transmitter granules obtained by high g centrifugation of press juice from bovine splenic nerves have been studied. Of the total noradrenaline present in the press juice 20—35 per cent was recovered in the sediment. The granules are stable for several hours at + 4° C but rapidly lose their noradrenaline on incubation in isotonic neutral media at 37° C. Noradrenaline is rapidly released even at low temperature at pH 4 and below and by detergents. Hypo- and hyperosmotic solutions and freezing and thawing had moderate releasing effect. Acetylcholine, nicotine, serotonin and GABA had no detectable effect on the noradrenaline release from the granules. Nerve granules are more sensitive to temperature than adrenal medullary granules but show higher resistance to freezing and thawing and osmotic changes.

High speed centrifugation of homogenates of bovine splenic nerves yields a sediment which contains noradrenaline in high concentration (EULER and HILLARP 1956). This finding has been confirmed by SCHÜMANN (1958) who also demonstrated the presence of ATP in the sediment, suggesting similar properties of the nerve granules as in those from adrenal medulla (FALCK, HILLARP and HÖGBERG 1956).

EULER and HILLARP (1956) further showed that the transmitter granules isolated from adrenergic nerves are relatively stable in isotonic solutions but readily give up their contents when exposed to acids.

The specificity of the granules was indicated by the fact that they did not contain histamine, which was present in large quantities in the original material. Chromatographic analysis of extracts of the separated granules gave no indication of the presence of other catechol compounds (EULER 1958), nor did biological tests reveal actions of any other kind than noradrenaline.

Previous experiments (EULER 1958) indicated that heating to 100° C, addition of acids and detergents cause a release of the noradrenaline contained in the particles.

In the present paper some results obtained with adrenergic nerve granules prepared by an improved technique will be described.

Methods

Bovine splenic nerves obtained at the slaughter house were dissected free from adjacent tissue and the nerve sheath removed. Preparations were made within 2 hours after the death of the animal. The nerves, usually 3—10 g, were collected on ice, wrapped in gauze cloth and squeezed between nylon cylinders. The press juice together with the washing fluid (see below), about 10 ml per g of nerve, was collected on ice and centrifuged for 5 min at about 1 000 × g and the sediment discarded.

As washing fluid for the primary suspension, sucrose in 0.3 M solution was used initially but later abandoned since it was observed that the yield was similar when sodium chloride or potassium phosphate were used as suspension media. Sedimentation in sucrose required higher g and longer centrifugation time; moreover sucrose interfered with the fluorimetric assay of noradrenaline.

Plasma has also been tried as suspension medium but did not seem to offer any advantages.

In the majority of the later experiments 0.075—0.15 M potassium phosphate of pH 7.2—7.3 was used as washing fluid for the primary suspension. This was then centrifuged for 30 min at about 5° C at 50 000 × g. The supernatant was adsorbed on alumina and eluted with acetic acid for fluorimetric estimation of noradrenaline according to EULER and LISHAJKO (1959).

After careful drying of the walls of the centrifuge tubes with moistened filterpaper the sediment was resuspended in various media. These were allowed to act for certain time periods and at different temperatures as described in the text. Thereafter the suspension was again centrifuged for 30 min in which time complete resedimentation was achieved. The supernatant was used for fluorimetric assay of noradrenaline.

Extraction of sediment

The noradrenaline present in the sediment could be quantitatively extracted with 0.1 N HCl added to give pH 1. By addition of 0.2 ml 2.3 M Na_2HPO_4 the granules could be sedimented by centrifugation at 10,000 g for 5 min. With HCl alone or with perchloric acid, sedimentation required longer time at high g. A lower yield was regularly observed when extraction was made with HCl at pH 0.5—0.7 than at pH 2. Trichloracetic acid was unsuitable for extraction of the granules since it interfered with the fluorimetric assay. When acid alcohol was used for extraction it was found that some activity remained in the precipitate.

The noradrenaline present in the extract of the granules was estimated by the fluorimetric method of EULER and LISHAJKO (1959).

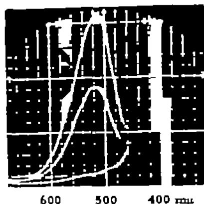


Fig. 1. Fluorescence spectra of extract of sediment from press juice from bovine splenic nerv (upper curve) oradrenaline (middle curv) and blank (lower curv) after transformation to lutines. Activating wavelength 395 mμ.

Results

Catechol compounds in supernatant and sediment from p ess juice of bovine splenic nerves

In a previous paper (EULER 1958) it was reported that chromatography of an extract from sediment obtained by high *g* centrifugation of press juice from bovine splenic nerves only revealed the presence of noradrenaline. If the catechol compounds present in extracts of the sediment were condensed w th ethylene diamine or oxidized to lutines and subjected to spectrophotofluori metric analysis (Aminco) evidence was obtained for the presence of noradrena line only (Fig 1)

In 4 experiments the supernatant from centrifugation of suspensions of bovine splenic nerve granules in 0.3 M glucose was passed through an alumina column and the catecholamines eluted with 0.25 N sulphuric acid. Th con

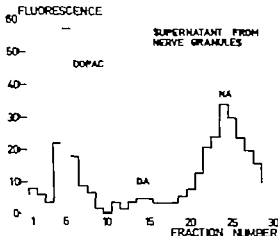


Fig 2. Starch column chromatogram of supernatant after high *g* centrifugation of microgranule suspension from bovine splenic nerves. Solvent n-butanol-acetic acid-n/10 HCl (5 1 1) Ordinate, fluorescence in arbitrary units after condensation with ethylene diamine Abscissa, fraction number DOPAC = 3 4 dihydroxy phenylacetic acid. DA = dopamine, NA = noradrenaline.

Table I. Total noradrenaline (NA) obtained from bovine splenic nerves by squeezing; percentage NA in the sediment and release during incubation for 30 min at + 20°C. Mean and S. D. from 15 exp.

Total amount of NA per g nerve obtained by squeezing	Mean recovery of NA in sediment in per cent of total	Mean release of NA on incubation 30 min + 20°C in per cent (controls)
8.7 ± 1.35 μg/g	28.5 % ± 4.5	33 % ± 7.6 %

centrated eluate was chromatographed on a starch column in a solvent system of n-butanol-acetic acid n/10 HCl (5 : 1 : 1). Fig. 2 shows the relative fluorescence of aliquots of the different fractions after condensation with ethylene diamine. The fluorescence peaks agreed well with those of dihydroxyphenyl acetic acid (fraction 4—6) and with noradrenaline (fraction 22—30). It was particularly noteworthy that the dopamine fluorescence peak was well marked only in 2 of the experiments, suggesting that there is no direct quantitative relationship between dopamine and the nerve transmitter.

Total yield of noradrenaline; recovery in sediment and release on incubation

The total amount of noradrenaline obtained from bovine splenic nerves by the squeezing technique was on an average 8.7 μg per g which compares well with the amount obtained by extraction with trichloroacetic acid and fluorimetric estimation (uncorrected). The results thus indicate that the yield was nearly maximal. In 15 consecutive experiments, in which the sediment after resuspension in 0.075 M potassium phosphate was incubated for 30 min at + 20° C, the percentage total recovery of noradrenaline in the sediment and the proportion released during incubation in the controls was estimated (Table I.

Effect of temperature and time of exposure on granules

At low temperature the granules retain their contents of noradrenaline for several hours. When a suspension made from bovine splenic nerves was prepared with 0.15 M KCl at pH 7.0 and centrifuged immediately the amount of noradrenaline per g of nerve in the sediment was 2.1 μg in two samples. In two other samples which were allowed to stand for 180 min at + 4° C after centrifugation the same amount was found. Even after 5 hours exposure at + 5° C at pH 7.7 the noradrenaline amount in the sediment was only insignificantly decreased.

In other experiments in which the suspension at pH 6.8 — 7.7 was exposed to + 4° C for 180—300 min before centrifugation, the noradrenaline content of the sediment was equal to that of the controls centrifuged immediately

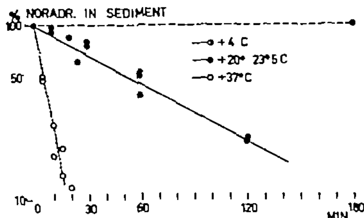


Fig. 3. Effect of temperature on noradrenaline release from isolated granules from bovine splenic nerves incubated in 0.15 M potassium phosphate or 0.15 M Na Cl at pH 6.8—7 7

(Fig. 3) The release on incubation at + 20° C proceeded at a faster rate so that 70—80 per cent of the activity was liberated after 2 hours exposure No difference was found with 0 15 M NaCl or 0 15 M potassium phosphate solution as incubation media. At 37° C the release proceeded at a much faster rate as seen in the figure.

Freezing and thawing

It was shown by HILLARP and NILSON (1954) that when chromaffin cell granules from th adrenal medulla were exposed to freezing and subsequent thawing a considerable portion of their amine content was released.

In a series of experiments the effect of freezing and thawing was studied on splenic nerve granules either in suspension or in the sediment after centrifuga tion. The samples were frozen by placing the original suspension or the re-suspended sediment in a deep-freeze at — 26 C, followed by thawing t room temperature. In some experiments the freezing and thawing was repeated up to 6 times. Controls were kept at + 3—5 C where the spontaneous loss was negligible. In all instances the releasing effect of this treatment was only moderate as illustrated in Table II.

As seen in the table the extra release caused by freezing and thawing reached 50 per cent only in one case in which the process was repeated 5 times with distilled water as suspension medium. In 0 15 M KCl repeated freezing and thawing had no more effect than a single treatment, and in 0.075 M potassium phosphate the loss from the sediment was 21 and 35 per cent compared with the controls. In those instances (exp. 4 and 5) where noradrenaline was also determined in the supernatant after recentrifugation the deficit observed in the sediment after freezing and thawing was found in the supernatant.

Table II. Effect of freezing and thawing on the release of noradrenaline from nerve granule suspensions

Exp. no.	Suspension medium	pH	Number of freezings and thawings	Percentage release
1	0.15 M KCl	6.7	1	23
2a	0.15 M KCl	6.7	6	28
2b	0.15 M KCl	7.0	1	19
3a	H_2O	6.8	1	20
3b	H_2O	6.8	5	50
4	0.075 M K-phosphate	7.6	1	33
5		7.0	1	21

Effect of osmolarity

During the initial stages of this investigation it was observed that the use of water as suspension medium gave a smaller yield of noradrenaline in the sediment than if isotonic solutions were used. The effect of varying osmolarity was therefore studied using KCl, NaCl and $CaCl_2$ as suspension media. The primary suspension was prepared with water and the sediment resuspended in the electrolyte solutions. The total time of exposure of the granules to the different media was approximately 5 min of preparation and 30 min of centrifugation at pH 6.0—6.4 at a temperature of about +3° C.

Fig. 4 shows the effect of solutions of different tonicity given in terms of osmolarity. In each series one sample was exposed to water as resuspension medium. The concentrations of isotonic solutions (0.3 osm) were approximated to 0.15 M for KCl and NaCl and 0.10 M for $CaCl_2$.

As seen in Fig. 4 maximal stability at pH 6.0—6.4 was observed in the region of 0.3 osmolarity, i.e. in isotonic solutions, while increased release occurred

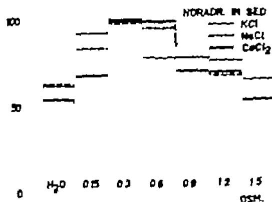


Fig. 4. Relative amount of noradrenaline in sediment after suspension in various media at different osmolarities. Exposure time 5 min., 30 min centrifugation. 4—5° C and pH 6.0—6.4.

Table III. Noradrenaline remaining in splenic nerve granules after incubation 30 min at + 20°C at pH 7.0 in 0.13 M potassium phosphate

Cetyl pyridinium bromide	10 mM	8.6 per cent of control
	1 mM	17
Sodium lauryl sulphonate	1 mM	7.8

in hypertonic as well as hypotonic solutions. The relatively large release in strongly hypertonic solutions is notable. Even when water was used as suspension medium the amount of noradrenaline in the resedimented granules still was about 50—60 per cent of that in the controls in isotonic solution, indicating a moderate degree of lysis only.

Membrane active compounds

A considerable release of noradrenaline from the sedimented granules into the supernatant after addition of detergents, such as cetyl pyridinium bromide and sodium lauryl sulphonate, to the suspension media, has previously been observed (Euler 1958). In new experiments the resuspended granules were incubated with detergents and the amounts of noradrenaline present in the sediment and the supernatant measured. Both detergents in a concentration of 1 mM caused an almost complete loss of amine content in the transmitter granules in 30 min at + 20° C as shown in Table III.

Incubation for 30 min at + 20° C with saponin (Merck) in concentrations of 1—30 mg/ml in the resuspended sediment at pH 7.0 in 0.075 M potassium phosphate did not alter the mounts of noradrenaline which were present in the sediment.

Ether in concentrations of 1 per cent in the primary suspension did not influence the yield of noradrenaline in the sediment.

Table II. Influence of various electrolytes on the noradrenaline content in granules. Temp. + 5°C., Time 95 min., pH 6.8

Medium	Conc. M	Tonicity (Isotonic = 1)	Noradr. in sediment µg/g nerve
NaCl	0.15	1.0	1.09
KCl	0.15	1.0	0.99
$CaCl_2$	0.10	0.85	1.12
$MgCl_2$	0.10	1.0	1.16
K-phosphate	0.075	(0.6)	1.12
Na-acetate	0.15	1.0	1.03
H_2O			0.84

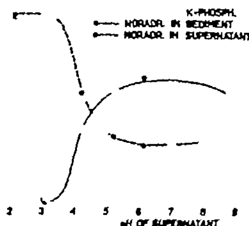


Fig 5. Noradrenaline in sediment and supernatant from resuspended granules from bovine splenic nerves at different pH. Incubation medium 0 075 M potassium phosphate, time 30 min at + 20° C. Ordinate, arbitrary units from zero.

Electrolyte suspension media

A comparison of the effect of various cations and anions on the rate of release of noradrenaline from granules at low temperature did not reveal any significant differences as shown by Table IV Nor did incubation for 30 min at + 22 C with the disodium salt of ethylene diamine tetraacetic acid cause any release of noradrenaline from the granules.

Effect of H concentration

It has been observed previously that add tion of acids to pH 5 or below caused a rapid release of the noradrenaline bound to the granules. In the present work the effects of pH on the release of the transmitter from the granular stores has been studied in more details. As suspension media water and various electrolytes were used to which acids and alkali were added to gi e the required pH The following solutions were used 0.15 M NaCl 0.15 M KCl 0.10 M $CaCl_2$, 0.10 M $MgCl_2$, 0.10 M K_2SO 0.075 M K-phosphate and 0.15 M sodium acetate

The granules showed good stability in the pH range of 5.5—8.8 w th only small differences in the noradrenaline content when phosphate was used as suspension medium. The somewhat narrower maximal tability range in the other media pH 6 0—8.5 may be due to the lack of buffering capacity of the media. For this reas n the pH of the suspension medium may have been temporarily higher or lower than the final pH of the suspension on add tion of acids or alkal A partial release may therefore have occurred initially

Complete loss of noradrenaline from the sediment was observed when the pH of the suspension was 3 5 and below In the pH range 3.5—5 3 a partial release was observed during the experimental conditions prevail ng

Fig 5 illustrates graphically the amounts of noradrenaline in the sediment and in the supernatant at d fferent pH alues in the suspension. The figure

shows that the sum of these amounts is constant for each sample indicating that all of the released amine is found in the supernatant.

A number of series were made with samples adjusted to pH 6.5—9 0 with narrower pH intervals in the region 7 0—8.0. Maximum stability was observed at pH 7.3—7 7 although the difference between the figures obtained in the region from pH 6.5—8.5 were small.

Effect of some autopharmacologically active agents

In some experiments the effect of various naturally occurring biologically active substances and some functionally related drugs have been studied after addition to a suspension of noradrenaline granules. After resuspension of the sediment in 0.075—0.13 M potassium phosphate and addition of the drug the suspension was incubated for 30 min at + 20 C. No effects were observed with acetylcholine, histamine, serotonin and GABA. Since serotonin seriously disturbed the analyses it had to be removed by selective adsorption of the noradrenaline on an alumina column. The dose ranges used were for acetyl choline 3.3—20 mg/ml, histamine 3—300 µg/ml, serotonin 0.2—10 mg/ml and for GABA 5—15 mg/ml. Nicotine 3—300 µg/ml was likewise without effect.

The actions of tyramine, dopamine, octopamine (kindly given to us by Prof V Erspamer) and phenylethylamine were studied in a special series the results of which have been previously reported (EULER and LISHAJKO 1960 a)

Discussion

The sediment obtained by high g centrifugation of a suspension prepared from adrenergic nerves by a simple squeezing procedure has proved to be useful for the study of a variety of factors on the storing ability of the microgranules. A number of observations support the assumption that these microstructures behave as if covered by a membrane having some properties in common with cell membranes. Thus the contents are readily released by addition of detergents or acids to a granule suspension. The loss of noradrenaline in hypertonic and hypotonic media and the maximal stability in isotonic solution likewise suggest a biomembrane.

That the particle bound noradrenaline is well protected is evident from the observations that manganese dioxide t pH 6 only inactivates the noradrenaline occurring free in solution. Furthermore the noradrenaline present in granules passes through an alumina column and can be released by addition of acid after the passage.

The mod of binding of the noradrenaline to the storage granules is still unknown. It has been shown by SCHÜMANN (1958) that nerve granules from adrenergic nerves contain ATP lik the chromaffin cell granules (FALCK et al 1956 BLASCHKO et al 1956)

While the total noradrenaline present in the press juice from bovine splenic

nerves in our experiments has been on an average about 9 μg per g, the amount recovered in the sediment has been about 2—3μg per g, or 20—35 per cent of the total. Whether the amount found in the supernatant occurs in a free form or is loosely bound to granules or some other cell constituent is at present not possible to state. The granular fraction shows a high degree of stability at low temperature but readily gives off the noradrenaline at higher temperatures. Especially the rapid release observed in a preparation of granules in isotonic potassium phosphate at + 37° C is hardly compatible with a storage function under the conditions prevailing in the isolated system. The observations suggest rather that the system is in some way incomplete or disturbed, so as to enhance the releasing process.

Although no effects were observed with the different electrolyte solutions used in the suspension medium this of course does not exclude actions of cations and anions on the noradrenaline binding mechanism.

Acid reaction had a very strong releasing action below pH 4 apparently by splitting the bonds keeping the amines fixed to some constituent of the specific structures. Even at pH 4—5 this effect was observed although it was less marked.

A number of drugs have previously been found to cause a release of noradrenaline from the granular sediment, among them tyramine and reserpine (EULER and LISHAJKO 1960 a, b, SCHÜMANN 1960) and local anesthetics in high concentrations (EULER 1960). In view of the stimulating effect of acetylcholine and other compounds on chromaffin cells and postsynaptic adrenergic neurons it appeared of interest to study the effect of this compound on the granules. The absence of effect with this substance or with nicotine or serotonin seems to indicate that their cell stimulating effects are not effectuated by an action on the intracellular granules themselves but must be due to triggering an indirect releasing effect.

Some quantitative differences were noted between the nerve granules and the adrenal medullary granules with regard to the releasing effects of temperature, hypotonic solutions, freezing and thawing and detergents. Thus HILLARP and NILSON (1954) found only about 10 per cent release in 10 min at + 45° C in adrenal medullary granules (0.3 sucrose) while the nerve granules in our experiments had lost about 80 per cent of the noradrenaline in 10 min at 37° C (0.13 M potassium phosphate).

On the other hand freezing and thawing had a much stronger action on the adrenal medullary granules than on the nerve granules (about 70 per cent release as against 20—30 per cent).

With adrenal medullary granules HILLARP and NILSON (1954) found that lowering of the molarity of the suspension medium from 0.25 M to 0.15 M sucrose for 1 hour at + 3° C caused an almost complete release of the catecholamines in the granules. Even suspension in water released less than 50 per cent of the noradrenaline in the nerve granules in the same time, however

The apparently larger effect of sodium lauryl sulphonate on nerve granules in comparison with medullary granules may be due partly to the smaller amount of material used in the experiments with nerve granules.

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Air Flow Patterns and Heat Transfer Within the Respiratory Tract

A new method for experimental studies on models

by

SVEN INGELSTEDT and NILS GUNNAR TOREMALM

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Abstract

INGELSTEDT, S. and N. G. TOREMALM. *Air flow patterns and heat transfer within the respiratory tract.* Acta physiol. scand. 1961. *51.* 204—217. — The authors have worked out an experimental method which makes it possible to determine air flow patterns in models of the respiratory tract. The method is based on the close relationship between heat transfer and pressure drop conditions which occur in a tube with a warm inner surface when air is blown or sucked through it. The air temperatures before entering and after leaving a tube of tracheal dimensions are determined and the heat transferred to the passing air is computed. Thus heat is used as a tracer. As a result of friction to flow an aerodynamic boundary layer is built up. It is stated that the shape of this layer determines the actual degree of heat transfer between the airway mucous membranes and the respired air. The hitherto unknown biological importance of variations in the boundary layer within different parts of the respiratory tract is briefly discussed.

Variations in the type of air flow that occurs within the human respiratory tract have an influence not only on the mechanics of breathing but also, more directly, on the interchange of heat and moisture between the respired air and the mucous membranes. An intense wall contact is physiologic and valuable within some regions of the upper airways, e. g. the nose and the subglottic space (INGELSTEDT and TOREMALM 1960). During special conditions, e. g. oral breathing of room air with low relative humidity or breathing through a tracheal



Fig. 1. The experimental device. 1. Thermocouple for measuring the actual room temperature. 2. The three variations of preconnected constriction. 3. Tracheal model with thermostat-regulated wall temperature. 4. Thermocouple for measuring the emerging air. 5. Electromagnetic valve. 6. Orifice meter. 7. Sucking fan regulated by an autotransformer.

stoma, however an extreme degree of wall contact may be deleterious to the easily damaged ciliary epithelium (TOREMALM 1960, 1961). Flow patterns with reference to the upper respiratory tract, mainly the nose, have hitherto been studied only with pressure difference methods (SEMERAK 1958, TONNDORF 1958 and others) and with the aid of either dye fluid (WEST and HUGH-JONES 1959) or smoke flow in model experiments (PROETZ 1953, BIRKMEYER 1939). The direct relation between pressure drop, flow patterns and degree of wall contact within the airways has not yet been thoroughly analyzed, which may be due to the lack of a sufficiently sensitive method for aerodynamic studies in short tubes (length less than 50 times the diameter) during intermittent flow and at physiological air flow rates.

In this paper a new method for such studies is described. It is based on the connection between aerodynamic and heat transfer phenomena appearing in a tube with warm walls when a gas — for example ordinary room air — is blown or sucked through it, whereby heat is used as tracer.

A tracheal model is tested in different manners well comparable with adult human airway conditions. The results are discussed from a physiological point of view while the clinical applications are described in another paper (INGELSTEDT and TOREMALM 1960).

Method

A double-walled brass tube (length 140 mm, diameter 22 mm) was used as a tracheal model (Fig. 1: 3). The wall temperature was kept constant at 36° C with the aid of circulating water from a thermostat. Air was intermittently sucked through the system 16 times per minute by an electric fan at flow rates of 20—60 l/min. The air flow was regulated with an electromagnetic valve and was determined by an orifice meter. The tube was coned in one end, where a quick-responding micro-thermocouple was mounted (Fig. 1: 4). The thermocouple was made of constantan and nickel-chromium wires with a diameter of 0.03 mm and soldered end to end so that the joints had the same diameter as the wires. This instrument, which is illustrated in detail in Fig. 2, measured the temperature of the emerging air.

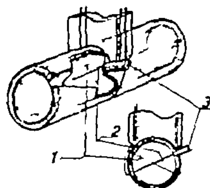


Fig 2 The authors micro-psychrometer in detail mounted in doubl -walled plexiglass tube (inner diameter 8 mm) 1 The "dry bulb thermocouple (The "wet bulb thermocouple (2) and the water upply channel (3) ere not used in the present experiments.)

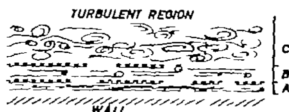


Fig 3 The fully developed boundary layer during turbulent flow (A) the buffer layer (B) and the central turbulent air stream (C)

The room air temperature was kept fairly constant at 24 C, but minor changes could be measured with a second thermocouple (Fig 1 1) placed near the inlet for the air flow

The cooled junctions of the thermocouples were immersed in ice (0° C The potentials arising in the hot junctions were converted to 50 /p alternating current w th the aid of Brown converters The output oltages were recorded with a direct ha nel ECG-apparatus (Mingograph, Type 230, ELEMA Ltd., Stockholm) For computations of the results see Koch *et al* (1958) and Fig 7

Thus by recording the air temperature before entering (t_{in}) and after leaving the tube (t_{out}) the heat transfer was calculated as described below

The following experimental series were performed

1) Increasing degree of contraction with the aid of preconnected smooth circular constrictions (Fig 1 2) at the free end of the mod l

2) Increase of entrance length with the id of added preconnected t bes of the same diameter as the model.

3) Increase of entrance length and a constant contraction (diameter 8 mm) at the free end of the system

4) Measurements of the pressure drop across t be with the same dimensions as the model during stead stat cond tions whereby sensitiv alcohol manometer was used (*0 mm alcohol column 1 mm H O

Fundamental properties of heat tran fer

There are three fundamental w y of heat transfer conduction, on ection and radiation. If the w rming object is moi as well, heat ma lso be transferred by me ns of condensation or ev poration (The la ter is not discussed in this paper

It may be assumed that the present experiment only con ection nd onduction are essential. If no air passes through the tube the en losed air will soon ttain the w ll temper ature i.e through onduction and free convection.

When air is blown through the tube however special aerodynamic phenomena are involved and the heat transfer is more intense and more complicated (forced convection) Part of the incoming air adheres to the wall because of friction forces and so-called laminar boundary layer is built up (Fig 3) Between this and the central turbulent air stream there is also buffer layer

At increasing flow velocities the temperature of the outgoing air gradually decreases until point is reached at which the temperature is sensibly constant and independent of further increment of the velocity It was suggested by REYNOLDS already 1874 that this state is reached at the "critical" velocity point where laminal flow is changed to turbulent one.

In spite of the turbulent flow which normally exists in the upper respiratory tract (GARAMLER, MALONEY and BJORK 1952) such a laminar region is, however still present near the wall. The thickness of this layer is mainly dependent on the air flow velocity

Within the boundary layer and the structure of the wall heat is transferred by conduction, which is relatively slow process as compared with heat convection. Therefore the thickness of the boundary layer practically determines the degree of heat transfer

Theoretical calculations

The heat transfer is characterized by the heat transfer coefficient (h) which is approximatively inversely proportional to the thickness of the boundary layer It is defined by Newton's Law of heat transfer as follows

$$Q = h \cdot F \cdot (t_{surface} - t_{surrounding\ air}) \quad (1)$$

where

Q = heat transfer per unit of time

F = surface area

$t_{surface}$ = surface temperature and

$t_{surrounding\ air}$ = temperature of the surrounding air

h = local heat transfer coefficient.

It may be observed that the heat transfer coefficient (h) varies along the tube. This variation depends on differences in structure and thickness of the boundary layer along the inner wall. As not only the boundary layer but also the main flow in the region of the tube inlet strongly depends on the contraction, it may be expected that also the heat transfer coefficient changes considerably at different degrees of inlet construction. The tests entirely confirm this assumption. As far as we know the heat transfer coefficient at different inlet constrictions has not yet been investigated and it may therefore be justified very briefly to make some suggestions as to this variation in the vicinity of the inlet.

In the very first part of the inlet there is no influence from disturbances and also no boundary layer It is, however necessary to assume a very high value of the heat transfer coefficient but it is of no importance whether this value tends towards infinity or whether it is finite (See Fig 4)

Immediately after the constriction there are more or less stationary whirls and therefore the exchange of heat between the wall and the central part of the flow is small. It may be assumed that the influence of these whirls decreases further on and therefore the heat transfer coefficient increases.

When finally the laminar boundary layer is built up to constant thickness the heat transfer coefficient becomes constant. It is difficult to predict if this final value is higher or lower than the value in the transient region, but it does not seem unreasonable to

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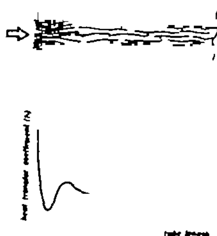


Fig. 4. The type of air flow in the original model (above) and in the model provided with an inlet constriction (below) in broad outline. I the middle the theoretical distribution of the heat transfer coefficient along the tube.

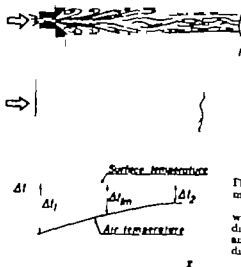


Fig. 5. The figure demonstra es how the logarithmic mean temperature differenc is computed. t_1 the emperature differenc between tube wall nd room air and t — the temperature difference between ube wall and the outpoint air t_{lm} logarithmic mean temperatur difference.

assume that the ariation is in accordance w th Fig. 4. I the upper and lower part of this figure the flow is sket hed in broad outline with nd without inlet constrictions.

With regard t the hoice of measurement method t is only possibl t determine the mean value of the heat transfer coefficient along the tube. The size of the nlet constriction and th length of the tube must therefore fluence this mean alue.

Besides the heat transfer coefficient the ir temperature lso varies along the tube. It is therefore necessary t introduce the well-know logari hmic-mean-temperature difference (Gröber 19 7 which is expressed by he follow ng equation See Fig. 5)

$$Q = k_m \cdot F \cdot \frac{\Delta t - \Delta t_1}{\ln \frac{\Delta t_1}{\Delta t}} = k \cdot F \cdot \Delta t_{lm} \qquad (2)$$

where

Q = heat transfer per unit of time.
k_m = average heat transfer coefficient.
F = inner surface area of the tube.
Δt_1 = temperature difference between tube wall and room air
Δt = temperature difference between tube wall and emerging air
Δt_{lm} = logarithmic-mean-temperature difference.)

From the equation k_m may be solved

$$k_m = \frac{Q}{F \quad \Delta t_{lm}} \tag{2'}$$

The amount of heat transferred from the wall per unit of time (Q) is the same as that beorbed by the passing air according to the following equation

$$Q = c_p \quad G \quad (t_{out} - t_{in}) \tag{3}$$

where

Q = heat transfer per unit of time,
G = mass velocity (mass per unit of time)
$t_{out} - t_{in}$ = mean temperature increase of the air and
c_p = specific heat (constant pressure).

The mass velocity is calculated as the product of flow rate (V) and mass density (ρ) as follows

$$G = V \quad \rho \tag{4}$$

Thus the average heat transfer coefficient (k_m) may be calculated from the equations 2' 3 and 4

$$k_m = \frac{\rho \quad c}{F} \quad V \quad \frac{t_{out} - t_{in}}{\Delta t_{lm}} \tag{5}$$

In this equation the following units can be used.

ρ in kg/m³
 in kcal/kg °C
F in m²
V in m³/h
t in °C.

Thus the average heat transfer coefficient (h_m) is expressed in kcal/m² h °C.

Example

The model provided with   constriction (diameter 5 mm)
Tube inner surface is 0.94 10⁻³ m wall temperature 36° C, olume flow 60 l/min, room air (t_{in}) 24.5° C and outgoing air (t_{out}) 31.0° C.
Mass density 1.15 kg/m³ and specific heat = 0.24 kcal/kg °C

$$k_m = \frac{1.15 \quad 0.24}{0.94 \quad 10^{-3}} \quad \frac{60 \quad 60}{1{,}000} \quad \frac{31 - 24.5}{7.8} = 88.0 \text{ kcal/m}^2 \text{ h °C}$$

) The logarithmic-mean-temperature difference can easily be computed with the aid of nomogram.

14

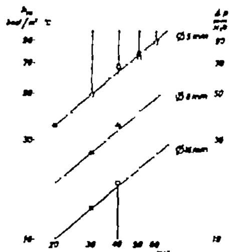


Fig 6

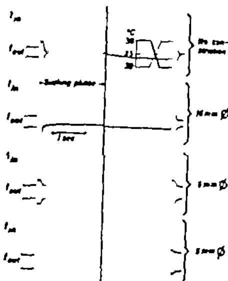


Fig. 7

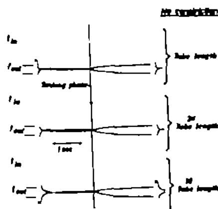
Fig 6. The computed heat transfer coefficients for the experiments with constrictions and the variations in air flow rate plotted on logarithmic scale in order to obtain a straight line relationship. The theoretical slope for the heat transfer coefficient (0.76) is drawn. □ = diameter 16 △ = diameter 8 mm and O = diameter 5 mm. The pressure drop values (●) obtained during continuous flow with the 5 mm constriction, showing slope closely related to the expected one which is 2.0.

Fig 7 Temperature recordings of room air (t_{in}) and the outgoing air (t_{out}) at flow rate of 30 l/min with increasing degree of constriction. Frequency 16/min. The sucking phase appears to the left in the recordings. The initial temperature rise is due to the considerable warming of the enclosed air during the silent phase (to the right in the recordings) when no sucking or blowing takes place The diagram plotted to the right abov show how the recorded temperature curves are computed. The zero line is shifted to 25°C and thus recorded temperature curve t 30 C has the same appearance as curve at 20° C.

Results

A. *Tracheal model with increasing degree of inlet constriction*

The following constriction diameters were used 16 8 and 5 mm. The constrictions correspond to a cross-sectional area of the human larynx, an ordinary tracheal cannula and a severe tracheal stenosis. The average heat transfer coefficients by increasing air flow rates are plotted on a double logarithmic scale in Fig 6. Temperature recordings at an air flow rate of 30 l/min appear in Fig 7 The temperature differences (t_{out} t_{in}) the average heat transfer coefficient (h_m) and the amount of heat transferred per unit of time (Q) from the wall to the passing air for all experiments in this series are given in Table I (mean value of three determinations)



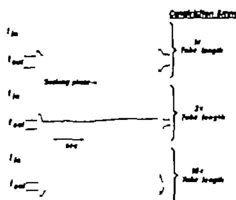


Fig. 8 Fig. 9

Fig. 8. Temperature recordings at flow rat of 30 l/min (frequency 16/min) with increasing degree of entrance length with the aid of preconnected tubes of the same diameter as the tracheal model.

Fig. 9. Temperature recordings at flow rate of 30 l/min (frequency 16/min) with increasing degree of entrance length and with constant constriction at the free end of the system (diameter 8 mm)

B. *Tracheal model with increasing degree of entrance length.*

A brass tube with the same diameter and with the same length (140 mm) as the model and another similar tube with a tenfold length (1 400 mm) were preconnected to the tracheal model. The temperature recordings at an air flow rate of 30 l/min appear in Fig 8. The temperature differences, the average heat transfer coefficients, and the heat transferred to the passing air in this series ppear in Table II (mean value of three determinations)

Table I. Temperature differences (t in C) heat transfer coefficients (h_m in kcal m² h C) and heat transfer per unit of time (Q in kcal h) with increasing degree of inlet constriction

Volume flow rate l min	Constriction diameter 16 mm			8 mm			5 mm			No constriction		
	$t_{out}-t_{in}$	h_m	Q	$t_{out}-t_{in}$	h_m	Q	$t_{out}-t_{in}$	h_m	Q	$t_{out}-t_{in}$	h_m	Q
60	2.25	31.9	2.2	3.75	42 0	3.7	6.50	88 0	6 5	0.25	2.2	0.2
50	2.50	30.5	2.1	4.00	36 6	3.3	6 50	73.5	5 4	0 00	0	0
40	2.75	18.8	1.8	4.75	33.6	3 1	7.25	67.0	4 8	0 00	0	0
30	2.75	14.2	1 4	4.50	26.2	2.2	7.00	49.3	3.5	0.25	1.1	0.1
20	3 00	10 4	1 0	5.25	21.5	1 7	7.25	35.0	2.4	0.25	0.8	0 1

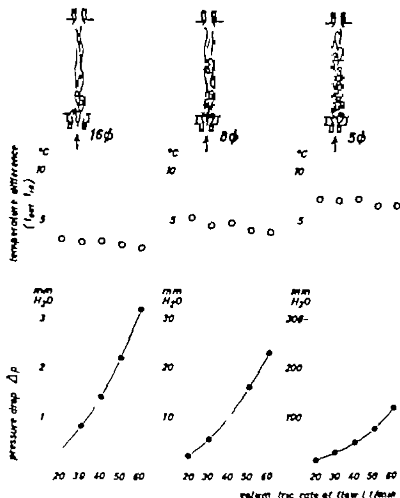


Fig. 10. The temperature increments at arying flow rates in experiments w th constrictions ppear in the diagrams abov Below the corresponding pressure drop values showing nonlinearity (turbulence

Table II Temperature differences (t in C) heat transfer coefficients (h_m in kcal m² h C) and heat transfer per unit of time (Q in kcal h) with increasing degree of entrance length

Volume flow rate l/min	Tube length × 1			2			× 10		
	$t_{out}-t_{in}$	h_m	Q	$t_{out}-t_{in}$	h_m	Q	$t_{out}-t_{in}$	h_m	Q
60	0.25	2.2	0.2	0.50	2.3	0.2	0.50	4.7	0.5
50	0.00	0	0	0.25	1.9	0.2	0.75	5.7	0.6
40	0.00	0	0	0.75	4.8	0.4	0.00	0	0
30	0.25	1.1	0.1	0.50	2.2	0.2	0.00	0	0
20	0.25	0.8	0.1	0.75	2.3	0.2	0.25	0	0

Table III Temperature differences (t in °C) heat transfer coefficient (h_m in kcal/m² h °C) and heat transfer per unit of time (Q in kcal/h) with increasing degree of entrance length and with constant inlet constriction

Volume flow rate l/min	Tube length 1			× 2			× 10		
	$t_{out}-t_{in}$	h_m	Q	$t_{out}-t_{in}$	h_m	Q	$t_{out}-t_{in}$	h_m	Q
60	3.75	42.0	3.7	2.00	20.4	2.0	2.50	27.0	2.5
50	4.00	36.6	3.3	2.25	19.4	1.9	2.75	24 7	2.3
40	4 75	35.6	3.1	2.25	13.8	1.3	3.00	21.8	2.0
30	4.50	26.2	2.2	2.25	11.8	1.2	3 00	16.3	1.5
20	5.25	21.5	1 7	2.50	8 6	0.8	3.25	11 7	1 1

C. *Tracheal model with increasing degree of entrance length and with a constant constriction (diameter 8 mm) at the free end of the system.*

The temperature recordings at an air flow rate of 30 l/min appear in Fig 9 The temperature differences, the average heat transfer coefficients, and the heat transferred to the passing air during this experimental series are given in Table III (mean value of three determinations)

D *Tracheal model with increasing degree of constriction compared with pressure drop determinations*

The pressure drops across the model w th preconnected constrictions were also determined (during steady state conditions) with a very sensitive alcohol manometer These results appear in Fig 10 where they are compared with the corresponding temperature differences (recorded during intermittent flow)

Discussion

The air flow within the human respiratory tract has hitherto mainly been considered from a purely mechanical point of view and little attention has been paid to air flow patterns and especially to the important direct contact that exists between the respired air and the mucous membranes. The latter conditions have not yet been investigated.

As regards air flow patterns in the nose, PROETZ (1953) has obtained very nice results with smoke in model experiments. Air flow patterns w thin the larynx have recently been studied in the same way by BIRKMEYER (1959) but only during continuous flow conditions. Furthermore none of these authors have reported what ir flow rate they used. WEST nd HUGH JONES (1959) have also made some experimental observations on flow patterns in a lung model. They studied the distribution of a dye in streaming water and in other experiments they injected argon with subsequent mass spectrometer analysis in gas samplings. As regards their experiments on inspiratory con-

ditions, however no attention was paid to the influence of the upper respiratory tract.

None of the methods mentioned yields satisfactory information on the very essential wall contact and thereby on the heat exchange that takes place between the respired air and the mucous membranes in different parts of the human respiratory tract. Therefore a more comprehensive knowledge concerning these problems is needed.

The present method — which is based on the close relation between pressure drop flow rate and heat transfer in a tube with uniform wall temperature — makes such studies possible. The recorded temperatures of the air before entering and after leaving the model yields simultaneous information on air flow patterns and the degree of wall contact during intermittent flow. These conditions can never be determined by the use only of smoke, dye or pressure methods.

The degree of heat transferred to the air after passing through the experimental device is due to the shape of the boundary layer because heat is transferred by means of conduction within the boundary layer and by means of forced convection between this layer and the free central air stream.

The chief object of the present study was

1 to test the method experimentally for comparison with theoretical calculations

2. to evaluate the effect of variations in air flow patterns within the human trachea on the basis of experimental data. The results, however also provide a reason for more general considerations regarding flow patterns in the whole respiratory tract.

*Ad 1 The reliability of the method appears from Fig 6 where the experi*mentally obtained slopes for the heat transfer coefficients and the pressure drops are seen to be almost identical with the expected theroretical ones.

Ad 2 Tracheal, bronchial and nasal aspects will be discussed one by one.

In the original model without constriction the critical velocity — the point at which a laminar flow becomes turbulent — was reached already at a flow rate of 30 l min, which may be theoretically calculated. Therefore it may be concluded, in accordance with GARSILER MALONEY and BJORK (1952) that turbulence normally prevails within the adult trachea even during quiet breathing at rest in both respiratory phases. In addition it is obvious (see Table I and Fig 7) that a turbulent type of flow may exist without any noteworthy direct contact between the wall and the airstream as the inspired room air passes nearly unchanged through the original model independently of air flow rates up to 60 l/min, i e. at a velocity far above the critical one.

Tracheal conditions

It must be remembered that no part of the respiratory tract, not even the trachea, can be compared with a straight tube. This is due to the fact that the larynx has an influence on inspiratory and the main bronchi on expiratory

flow patterns. The former factor is not considered in the experiments made by WITT and HOOK JONES. It is variations in the type of the entrance region itself and not in the entrance length that are essential. (See Table II and Fig 8.) In spite of a preconnected tube (length 1 4 m) hardly any heat exchange took place within the model. The constriction with 16 mm diameter has a cross-sectional area closely related to that of the adult human larynx during "inspiration" Inspiration" through this constriction compared with "inspiration" without any constriction (Table I and Fig 7) illustrates the increase in degree of wall contact distally to the constriction in the former case. The temperature of the emerging air increased by about 2—3 C which is in sharp contrast with the very small pressure differences obtained with a sensitive alcohol manometer The direct comparison between heat transfer and pressure drop is illustrated in Fig 10

From this it may be concluded that the human larynx functions as a constriction in an aerodynamic sense.

With increasing degree of constriction — as in the case of tracheotomy and pathological processes in the airways (equivalent to the constriction diameters 8 and 3 mm) — the degree of wall contact also increases and the region distally to the constriction is always the most exposed one (see Fig 3) Therefore, during inspiration the mucous membrane may be damaged by drying effects in that region. This is normally not the case as the inspired air is previously warmed and moistened to a very great extent in the nose. During prolonged oral breathing, however this disadvantage may occur During expiration no drying takes place but naturally the degree of heat and moisture recovery is influenced by constricting processes. It must be observed that the temperature differences in this experimental series were not affected by variations in flow rates from 20 to 60 l/min but naturally the amount of heat transferred to the air is trebled which results in ncreased demands on the mucous membrane.

BRISCOE, FORSTER and COMROE (1954) have made some experiments with inhalation of helium and oxygen. They found that gas portions considerably less than the normal dead space were able to penetrate the dead space and to reach the alveolar level which suggests a laminar cone front. This statement, however need not necessarily be irreconcilable with the present ones as these authors used 80 per cent helium, which has a far lower density than air and thus decreases or eliminates the turbulence.

Bronchial conditions

In their experiments on a lung model WITT and HOOK JONES (1959) found that there was a laminar type of flow in the trachea during the inspiratory phase up t a flow rate of 10 l of air per minute. At high flow rates (40 l/min) eddying was seen at the main carina and other bifurcations. From our experiments with preconnected straight tubes and a constant constriction (8 mm) t the free end of the system it ppears (see Table III and Fig 9) that the turbulence that

arises distally to the constriction extended throughout a length of 1.4 m. Therefore it seems to be possible that, in the human trachea (larynx included) turbulence exists even at a flow rate of 20—30 l/min and that the unsteadiness in the air flow proceeds below the main carina as the tracheal cross-sectional area is nearly equal to that of the main bronchi (JEMERIN and MEREZNOIKO 1957). This is however of little importance for the mucous membranes in this region since the inspired air is conditioned to about 80 per cent (INGELSTEDT 1956) already at the level of the larynx.

Nasal conditions

A very intense wall contact and a relatively thin boundary layer must be expected to occur in the nose during normal conditions. This assumption naturally presupposes a turbulent type of air flow in both directions but probably to a greater extent during the inspiratory phase. After studying the investigations of SEMERAK and of SEEBOHM and HAMILTON TONNDORF (1958) concluded that *nasal air flow during normal breathing is at the verge of eddying* or becoming turbulent. Pressure drop determinations across only one nasal cavity are unphysiological but on the other hand when they are made across both nasal cavities, they are very small and thus unreliable. Furthermore the following informations make likely a turbulent flow in the nose a) irregular inner configuration, b) narrower cross-sectional area in the vestibuli nasi as compared with that of the present model, where turbulence appeared already at a flow rate of 30 l/min and c) rapid warming of inspired air as shown by INGELSTEDT and TOREMALM (1960). (During breathing in a cold chamber at —12° C the inspired air had a temperature of + 25° C already when it reached the epipharynx.)

Conclusions

From the present experiments and considerations the following conclusions can be drawn.

1. Air flow studies in the respiratory tract are of great interest if they also yield information on the degree of contact between the mucous membrane and the respired air.

2. The shape of the boundary layer — built up through friction to flow — *is very important and determines* the degree of wall contact and hereby also the degree of heat transfer to the inspired air.

3. A turbulent type of flow exists during both respiratory phases in the nose, trachea and main bronchus during normal conditions.

4. From a functional point of view the human larynx is the narrowest region of the entire respiratory tract and functions as a constriction in an aerodynamic sense. The inspired air from nose and mouth is mixed in the subglottic space, where consequently the boundary layer is disturbed. *This may be of importance* at prolonged oral respiration and in pathological conditions.

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On the Origin of Lithocholic and Ursodeoxycholic Acids in Man

Bile Acids and Steroids 106

By

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Abstract

HELLSTRÖM, K. and J. SJÖVALL. *On the origin of lithocholic and ursodeoxycholic acids in man.* Acta physiol. scand. 1961. 51. 218—223. — Chenodeoxycholic acid-24 [illegible] C was given perorally to two healthy persons and intravenously to one patient having [illegible] bile fistula. [illegible] the normal subjects bile was collected from the duodenum every morning for three days. Only a minor part of the chenodeoxycholic acid had been metabolized. About 3 per cent of the activity recovered from the healthy subjects was present as lithocholic acid. Ursodeoxycholic acid accounted for [illegible] per cent of the activity in one of these subjects. None of these acids were found in the bile fistula patient. Unidentified labelled compounds more polar than chenodeoxycholic acid were present in all bile samples.

Lithocholic and ursodeoxycholic acids have been isolated in small amounts from human bile and lithocholic acid has also been found in human feces (SOBOTKA 1937, SJÖVALL 1959, MOSETTIG *et al.* 1958). In rats and rabbits these acids can be formed from chenodeoxycholic acid (SAMUELSSON 1959, MATSCHINER *et al.* 1958, NORMAN *et al.* 1960, HELLSTRÖM *et al.* 1960). Since chenodeoxycholic acid is one of the main bile acids in human bile, knowledge of its metabolism is important in quantitative studies of bile acid turnover. In order to see whether lithocholic and ursodeoxycholic acids can be formed in man during the enterohepatic circulation of chenodeoxycholic acid we have given chenodeoxycholic acid-24-[illegible]C to two healthy subjects and one patient with a bile fistula. The results are presented in this paper.

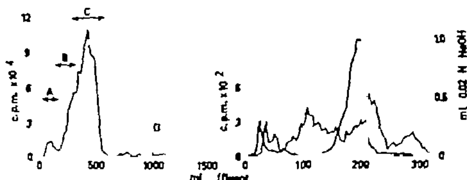


Fig. 1. Left curve: Chromatography of labelled compounds in hydrolyzed bile collected from the duodenum after administration of labelled chenodeoxycholic acid to healthy subject. Column: 45 g hydrophobic Supercel, phase system F 1. Right curve: Rechromatography of peak A (left curve) with phase system C 1. Column: 9 g Supercel. Solid line: Titration. Dotted line: Radioactivity.

Experimental

Chenodeoxycholic acid-24-C with a specific activity of 10 μC per mg was synthesized according to BERGSTRÖM *et al.* (1955). Ursodeoxycholic acid, m. p. 202—203, was synthesized according to SAMUELSSON (1960). Two young healthy female subjects were given 3.7 μC of the labelled acid *per os*. Fasting bile was collected every morning for three days. The samples were obtained from the duodenum through a polyvinyl tube after the intravenous injection of cholecystokinin-pancreozymin (LESHOFFEDT 1957) and the collection was continued as long as there was a flow of dark coloured bile.

In addition to the two normal subjects the labelled acid was given to one female patient, 59 years old. A T-tube had been inserted into the common bile duct following the removal of gallstones. Her liver function tests were normal and no bile pigments were present in the feces at the time of the experiment. Bile was collected for 12 hours after the intravenous injection of the labelled acid.

Extraction and chromatography. The bile samples were added to about 10 volumes of ethanol. After filtration and evaporation of the ethanol the bile acids were hydrolyzed in 1 N NaOH at 120° C for 6 hours. They were then extracted with ether after acidification of the aqueous solution. Reversed phase chromatography was performed as previously described (SJÖVALL 1953, NORMAN 1953).

The following solvents were used.

Phase system	Moving phase (ml)	Stationary phase (ml)
C 1	Methanol-water 150 : 150	Chloroform-isooctanol 15 : 15
F 1	Methanol-water 165 : 135	Chloroform-heptane 45 : 5
F	Methanol-water 180 : 120	Chloroform-heptane 45 : 5

Hydrophobic Hyflo Supercel was used as the inert support for the stationary phase.

Radioactivity was determined by counting of an infinitely thin layer on aluminium planchets in a Frieseke Hoepfner (FH 51) methane gas-flow counter.

Results

Normal subjects. A total of 14 and 26 per cent of the radioactivity was recovered from the duodenum of the respective subjects after the administration of the labelled chenodeoxycholic acid. Chromatography of the hydrolyzed bile

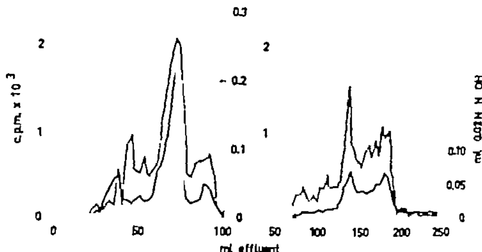


Fig 2. Left curv Second rechromatography of band B (Fig 1 left curve) together with unlabelled ursodeoxycholic acid. Column 9 g Supercel, phase system F 1 Right curve Rechromatography of band D (Fig 1 left curve) together with unlabelled lithocholic acid (see results) Column 9 g Supercel, phase system F 2. Solid lines Titration. Dotted lines Radioactivity

phase system F 1 showed that only 9 per cent of the chenodeoxycholic acid had been transformed in both subjects. The result of the analysis of the bile from one subject is shown in Fig 1 (left curve) As seen, a small peak of radioactivity appears with the front (A) The main peak has the same elution characteristics as pure standard chenodeoxycholic acid. Ursodeoxychol c acid is eluted from the columns slightly faster than chenodeoxycholic acid and if a small amount of labelled ursodeoxycholic acid were present it would be expected to elute with the first part of the large chenodeoxycholic acid peak. This peak was therefore divided into two parts which were rechromatographed separately (B and C) The fractions collected after the chenodeoxycholic acid peak were combined (D) *for further chromatographic analysis. Column* chromatographic analysis of the bile from the second subject was similar to the first and the fractions were treated in the same way

Band A (Fig 1) was rechromatographed with phase system C 1 and the result was identical for both subjects (Fig 1 right curve) No peak of radioactivity was found with the titration peak of the cholic acid originally present in the bile (Fig 1 right curve, 150—210 ml effluent) At least three radioactive compounds not identical with cholic acid were present.

Band B (Fig 1) was rechromatographed with phase system F 1 and the fractions thought to contain ursodeoxycholic acid were combined These fractions were again rechromatographed after addition of unlabelled ursodeoxycholic acid. No labelled compound *was eluted with the ursodeoxycholic* acid peak in one of the subjects. However labelled material was found in the

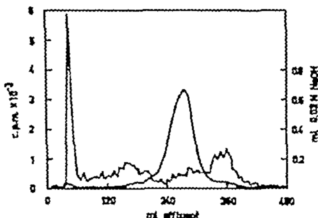


Fig. 3. Chromatography of labelled compounds more polar than chenodeoxycholic acid obtained from the bile fistula patient during 12 hours following the intravenous administration of chenodeoxycholic acid-24-^{14}C. Column: 13.5 g Supercel, phase system C 1. Solid line: Titration. Dotted line: Radioactivity.

ursodeoxycholic acid peak of the second subject as shown in Fig. 2 (left curve). The small peaks of radioactivity on both sides of the ursodeoxycholic acid peak (60—80 ml effluent) were due to the incomplete removal of chenodeoxycholic acid and the more polar compounds present in band A (Fig. 1). The identity of the labelled compound with ursodeoxycholic acid was established by isotope dilution.

Most of the radioactivity both in band B and band C (Fig. 1) coincided with the titration peak of the chenodeoxycholic and deoxycholic acids from the bile. It was therefore regarded as unchanged chenodeoxycholic acid.

The combined fractions designated D (Fig. 1) were rechromatographed with phase system F 2 after addition of unlabelled lithocholic acid. Radioactive peaks coinciding with the titration peak of the carrier were found in both experiments. One of the chromatograms is shown in Fig. 2 (right curve). Unfortunately crystallization of lithocholic acid on the column resulted in the appearance of a double peak. This is not commonly seen with bile acids other than lithocholic acid. The identity of the labelled compound with lithocholic acid was established by isotope dilution.

Bile fistula patient. To avoid influences of the intestinal flora on the metabolism of the chenodeoxycholic acid, the labelled acid was given intravenously to the patient with a T-tube in the common bile duct. Although the feces were acholic, passage of bile into the duodenum could not be completely excluded. Therefore only the bile excreted during the first 12 hours after the injection was used for the analysis of the labelled compounds formed. During this time about 25 per cent of the activity injected was recovered. Chromatography of the hydrolyzed bile showed that about 15 per cent of the activity was present as compounds more polar than chenodeoxycholic acid. The rechromatography of these compounds with phase system C 1 is shown in Fig. 3. The chromatogram is similar to the one obtained with the bile of the normal subjects (Fig.

I right curve) No peak of radioactivity coincided with the cholic acid titration peak (about 200—300 ml effluent) Rechromatography of the fractions that would have contained ursodeoxycholic and lithocholic acids respectively did not reveal the presence of these acids.

Discussion

The results obtained indicate that chenodeoxycholic acid is less extensively transformed in man than in rats, mice, pigs and rabbits. In rats and mice the 3α, 6β 7α- and 3α, 6β 7β-trihydroxycholanic acids are the main metabolites and these acids are formed from chenodeoxycholic acid in the liver (Hsia et al 1958, Samuelsson 1959 Danielsson et al. 1959) In pigs the intestinal flora is of great importance for the metabolism of chenodeoxycholic acid. This acid is 6α-hydroxylated in the liver to hyocholic acid which is then transformed into hyodeoxycholic acid by intestinal microorganisms (Bergström et al 1959) This transformation involves the removal of a 7α hydroxyl group in analogy with the formation of deoxycholic acid from cholic acid and of lithocholic acid from chenodeoxycholic acid (Lindstedt et al 1957 Bergström et al. 1959, Norman et al 1960) Removal of the 7α-hydroxyl group from cholic and chenodeoxycholic acids appears to be a dominant reaction in the bile acid metabolism of the rabbit (Lindstedt et al 1957 Hellström et al. 1960)

In the two normal human subjects studied bile was collected every morning for three days after the administration of labelled chenodeoxycholic acid. The combined bile samples from each of the two subjects contained about 3 per cent of the radioactivity as lithocholic acid. This acid was not to be found in the patient having a bile fistula indicating that the lithocholic acid is formed by intestinal microorganisms as is the case in the rat (Norman et al. 1960) Acids behaving similar to, but not identical with cholic acid were found in all three subjects. The chromatograms of these compounds were similar and it is therefore possible that they were formed in the liver Two per cent of the radioactivity in the bile of one of the normal subjects was present as ursodeoxycholic acid. This acid was not found in the other subjects but it is known that ursodeoxycholic acid cannot be found in all human bile samples (Sjövall 1959)

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Acta physiol. scand. 1961 51 224—229

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Tyramine Effects on Catechol Amine Release from Spleen and Adrenals in the Cat

By

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Abstract

STJÄRNE, L. *Tyramine effects on catechol amine release from spleen and adrenals in the cat* Acta physiol. scand. 1961 *51* 224—229 — Recent studies on the sympathomimetic effect of tyramine suggest that this substance may act by liberating noradrenaline In the present experiments direct bioassay of the venous effluents from the spleen and the adrenals of the cat demonstrated that the noradrenaline level in splenic vein blood was elevated by tyramine, whereas in the adrenal venous blood both the adrenaline and noradrenaline levels were either unchanged or depressed. The discrepancy between the effects of tyramine on these two systems is discussed. It is concluded that in the spleen tyramine may act either by releasing noradrenaline from the intra-axonal storage granules, or by making extragranular noradrenaline, bound to some structure probably in close relation to the axone, a ailabl t the receptors.

The observations of BURN and TAINTER (1931) that tyramine lost ut dilator effect on the cat s iris after denervation by removal of the superior cervical ganglion one or two weeks previously and of *BURN (1932) that the vasoconstrictor* effect of tyramine in the cat s foreleg was reduced or abolished after similar removal of the stellate ganglion, suggested that the sympathomimetic action of tyramine is dependent on intact sympathetic innervation.

In 1953 FLECKENSTEIN proposed as one possible interpretation that tyramine, as well as other sympathomimetic amines having two hydrogen atoms at the β-carbon in the side chain and either no hydroxyl group in the ring or one such group in para position, might exert its action by *releasing noradrenaline.*

The recent finding, that the vasoconstrictor action of tyramine is strongly reduced after the sympathetically innervated organs have been emptied of most of their catecholamines by reserpine treatment (CARLSSON *et al.* 1957) as well as the observation, that its normal action can be restored by noradrenaline and to some extent by adrenaline infusions intravenously have been regarded as further evidence that tyramine acts by releasing noradrenaline (BURN and RAND 1958)

In vitro experiments have demonstrated that tyramine liberates catechol amines from isolated storage granules, both from splenic nerves (EULER and LISHAJKO 1960, SCHÜMANN 1960) and adrenals (SCHÜMANN 1960)

The present experiments have been designed to study the action of tyramine on the adrenal medulla and the spleen of the cat, by collection of the venous effluent and direct determination of its catechol amine content, following tyramine administration.

Methods

Cats weighing 2.5—5.0 kg and anesthetized with Nembutal 35 mg/kg i.p., were prepared for collection of adrenal vein blood according to the method of SCHAPIRO and STYLANDER (1958) Heparin was given as anticoagulant, 4 mg/kg i. [illegible] initially and half this dose each hour

The adrenal vein blood was collected in graduated centrifuge tubes. After centrifugation at 1000 g for 5—10 min, hematocrit and volume were determined and the plasma withdrawn and stored in freeze-box for subsequent bioassay

In order to study the relationship of vasoconstriction in the spleen to "overflow" of noradrenaline in the venous effluent, the following procedure was used A clamp was placed across the middle part of the spleen, and one half of it was perfused with arterial blood from the left femoral artery of the same cat by a Sigma motor pump, giving a constant flow rate. The arterial blood pressure in this circuit, in the present report referred to as splenic arterial pressure, as well as the systemic arterial pressure, heart rate and respiration were recorded on Grass polygraph, using commercial transducer equipment. The venous effluent from the spleen was collected intermittently according to the same principles as those used for the adrenal vein blood. The main vein was ligated 2—4 cm from the organ and a polyethylene catheter was introduced into the vein, pointing towards the spleen During collection periods the blood was diverted through T-tube into graduated centrifuge tubes, whereas in the intervals it was returned to the cat via the right femoral vein. After centrifugation, hematocrit and volume determination and separation from the cells, the plasma was kept in a freeze-box for later catechol amine determination. For differential assay of adrenaline and noradrenaline in the adrenal vein blood, the cat's blood pressure and the hen rectal caecum were used. Pressor activity in splenic vein blood was determined on the blood pressure of the cat, premedicated according to KAERS (1956) No effort was made to distinguish between adrenaline and noradrenaline in the splenic effluent since it is known that more than 90 procent is noradrenaline (PEART 1949

The effect of tyramine on the secretion from the adrenals was studied before and after denervation by splanchnicotomy

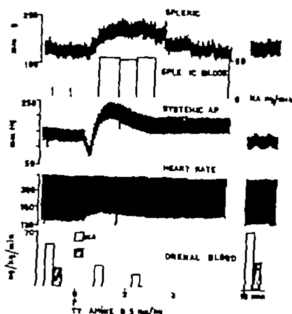


Fig. 1 The effect of 0.5 mg/kg tyramine intravenously on splenic arterial pressure, noradrenaline output from the spleen, systemic arterial pressure, heart rate and adrenaline and noradrenaline secretion from the innervated adrenal.

Results

Tyramine 0.5—1 0 mg/kg i v in the cat caused a powerful pressor response, occasionally preceded by a depressor phase, and a sometimes extreme tachy cardia, after pretreatment with atropine, 6 mg/kg intraperitoneally

The cardiovascular responses to repeated doses of tyramine showed a rapid decrease in cats treated with reserpine, 5 mg/kg i v 1—2 hours earlier The initial response was partially restored after i. v infusion of 15 μg/kg/min of noradrenaline for 5 min, after the blood pressure had returned to the preinfusion level.

The adrenal medullary secretion showed no increase during the peak of the pressor response to tyramine. On the contrary it was clearly depressed in most cats with intact adrenal innervation. This depression was not seen after adrenal denervation by splanchnicotomy After the peak of the cardiovascular response blood pressure and heart rate often failed to return to the resting level for about 10 min. During this phase there was a slight increase of adrenaline and noradrenaline in the adrenal vein blood

A further attempt to stimulate adrenal medullary secretion with tyramine was made by perfusing an isolated cattle adrenal according to the venous perfusion method of *Hecker et al.* (1953) with oxygenated heparinized blood from the same animal. No definite increase in the catechol amine content of the effluent was seen as a result of an infusion of tyramine, 10 mg/min for 10 min.

The resting noradrenaline level in the splenic vein blood was close to the

threshold of the present assay method and is represented in Fig. 1 as being less than 2.5 ng/ml. During the vasoconstrictor phase in the spleen following tyramine pressor activity in splenic vein blood rose to 6—8 ng/ml.

Discussion

The increase in pressor activity in the splenic vein blood in the cat caused by tyramine — as a sign of an increased "overflow" of noradrenaline — confirms the hypothesis of FLECKENSTEIN (1953) that tyramine exerts its sympathomimetic action by causing a local release of noradrenaline.

Similar observations have recently been published by LOCKETT and EAKINS (1960). They found that tyramine caused an increase both in the adrenaline and the noradrenaline concentrations of plasma from the aorta but not from the vena cava of adrenalectomized, hexamethoniumtreated cats. Their conclusion is that tyramine liberates both adrenaline and noradrenaline either from some stores in the arterial walls, or from the red cells.

The adrenals do not contribute to the immediate cardiovascular effects of tyramine, in the dosage used. This is in agreement with the results reported by STRÖMBLAD (1960) obtained with a different technique. The inhibition of the adrenal medullary secretion during the peak of the hypertensive response seen in most of the experiments did not occur after adrenal denervation and is thus probably reflex in origin. This is in accordance with the experiments reported by SCHAEPDRYVER (1959) who found inhibition of the adrenal medullary secretion when the isolated perfused carotid sinus of the dog was exposed to a local elevation of pressure, relative to the systemic pressure.

The small increase in adrenaline and noradrenaline secretion from the adrenals seen after the peak of the pressor response possibly indicates some contribution from the adrenals to the persistence of a slight elevation of blood pressure and heart rate, as compared to resting levels. However this increase may well be a reflexly mediated "rebound" phenomenon, since it does not seem to occur after adrenal denervation.

The absence of any definite sign of catechol amine release from the isolated perfused cattle adrenal, even with a very high dose of tyramine, may be regarded as additional evidence that the intact adrenal medulla is very insensitive to the amine liberating effect of this agent.

The present results indicate that the effect reported by SCHÜMANN (1960) that tyramine incubation accelerates the spontaneous release of catechol amines from isolated storage granules from cattle adrenals does not operate on the intact organ.

On the other hand, the liberation by tyramine of noradrenaline in vitro from isolated storage granules from splenic nerves in cattle (EULER and LISHAJKO 1960, SCHÜMANN 1960) may well explain the release of noradrenaline from the cat's spleen, obtained in the present experiments with tyramine.

The striking discrepancy between the catechol amine releasing effect of tyramine in the spleen and the adrenals might possibly be explained in terms of differences in accessibility to tyramine of the storage granules, in the two types of organ. Or else there may be a difference in the sensitivity to tyramine of storage granules from nerve tissue and chromaffin cells, as actually indicated by the differences in threshold concentrations for the releasing effect of tyramine observed in vitro (EULER and LISHAJKO 1960 SCHÜMANN 1960)

However the fact that even a very high dose of tyramine does not release any appreciable amount of catechol amines from the adrenal, whereas a small dose is an effective releaser of noradrenaline from the spleen, suggests a different explanation

The restoration by an i.v. infusion of noradrenaline of the gradually decreasing pressor response to tyramine after reserpine treatment observed in the present investigation, as well as several similar observations in the literature, notably by BURN and RAND (1958) indicate the existence of a storage mechanism for circulating catechol amines.

In contrast to the intraaxonal storage granules, this store appears to be characterized by a very limited uptake capacity judging from the fact that uptake has been possible to demonstrate only by the sensitive radioactive isotope methods (AXELROD 1960) Yet it is functionally important, since it restores the effects, not only of tyramine but of sympathetic nerve stimulation as well (ROSELL 1959 BURN and RAND 1960) after these have been abolished by *reserpine treatment* The implication of this appears to be that the postulated storage structure is closely related to the nerve fibre, possibly even located on the surface of the axone

If it is assumed that the sympathomimetic action of tyramine consists in releasing noradrenaline from a storage structure of the abovementioned type, related to the postganglionic sympathetic neurone but not to chromaffin cells, the discrepancy between the effects of tyramine on the spleen and the adrenals would seem to be explained.

The observation of tachyphylaxis in the blood pressure responses to tyramine less than two hours after reserpine, as well as the partial restoration of the tyramine response by a noradrenaline infusion are in agreement with the findings of BURN and RAND (1958) In normal cats the blood pressure response to tyramine remains unchanged for hours, whereas in the experiments described here a rapid decline was seen during minutes after reserpine. Thus reserpine evidently interferes with the resynthesis and/or storage capacity for noradrenaline.

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Respiration and State of Wakefulness in Normals, Studied by Spirography, Capnography and EEG

A preliminary report

By

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Abstract

BÜLOW, K. and D. H. INGVAR. *Respiration and state of wakefulness studied by spirography, capnography and EEG.* Acta physiol. scand. 1961. 51. 230—238. — In sixteen normal subjects the relation between the state of wakefulness and the respiration was studied by recording continuously the ventilation (spirography), the alveolar carbon dioxide tension (capnography) and the EEG. Records were obtained in the resting state awake, in drowsiness and, in nine of the subjects, in sleep and during arousal. It was confirmed that ventilation decreases and that the alveolar carbon dioxide tension increases in sleep (MAGNUSSEN 1944). When the EEG showed a stable pattern of [illegible] alpha-activity or sleep waves, the breath to breath variations in ventilation and alveolar carbon dioxide tension were small at respective levels. There was in all the sixteen cases a close correlation in time between sudden changes in EEG and changes in the respiratory variables recorded. The findings suggest a close functional relationship between the respiratory centers of the brain stem and the reticular activating system which supposedly controls the cortical state of excitability.

In his monograph on respiration in sleep, MAGNUSSEN (1944) clearly demonstrated the difference between wakefulness and sleep as to average respiratory minute volume and alveolar carbon dioxide tension. In sleep average ventilation is generally decreased and there is an increased carbon dioxide tension in the alveolar air as compared with the wakeful state. Furthermore in sleep there is usually a characteristic change in form of the individual breath as recorded in a respiratory valve at the mouthpiece by a pressure sensitive device.

Alveolar carbon dioxide concentration has been further studied by ROBIN et al. (1958) and BIRCHFIELD, SIEKER and HEYMAN (1959) who like MAGNUSSEN (1944) found a decreased sensitivity to carbon dioxide during sleep in normals. REED and KELLOGG (1958) investigated respiration in sleeping subjects at sea level and after acclimatization to high altitude. When falling asleep there was a definite increase in threshold value of ventilatory response to carbon dioxide at both altitudes.

In the present investigation we have recorded the EEG continuously in normal subjects which spontaneously passed through the stages of wakefulness, drowsiness, sleep and arousal. At the same time, respiratory functions were registered by means of continuous spirometry as well as continuous infra-red analysis of the carbon dioxide content of the ventilatory air (capnography). The "end-plateau" value of each expiration was taken to represent the alveolar carbon dioxide tension (P_{ACO_2}). In this way it was possible to study the gross time relationships between changes in the EEG and in respiration when changes in wakefulness took place. It will be shown that sudden EEG changes during shifts in the state of wakefulness were accompanied by simultaneous rapid alterations in ventilation as well as in alveolar carbon dioxide tension. The results suggest an intimate functional relationship between those parts of the brain stem which determine the cortical excitatory state (as reflected in the EEG) i. e. the so called reticular activating system (MAGOUN 1958) and those commonly designated as the respiratory centers.

Material and Methods

The investigation was carried out in the mornings in sixteen normal subjects (ten females and six males) aged 22 to 60, most of these being nightworkers at the hospital and accustomed to going to bed at the time of the recording period. All persons had undergone routine EEG as well as chest radiography and complete analysis of respiratory functions (including determination of the different lung volumes and capacities, the resting minute volume, the maximal voluntary ventilation, the oxygen consumption and the distribution of the ventilatory air and its relation to the pulmonary blood flow). All subjects showed normal EEG and pulmonary conditions and there were no signs of bodily or mental disorder.

Recording was carried out in a dark quiet room with suitably constant temperature. EEG electrodes were mounted symmetrically over the parieto-occipital regions on both sides of the scalp, 3 cm from the midline, and a continuous "bipolar" record was obtained in two leads by means of an ELEMA EEG-recorder (time constant 0.1 sec). In some cases the electrocardiogram was recorded by two electrodes placed on the right and left arm.

The subject to be investigated was lying comfortably in a reclining position and was allowed to breathe through a rubber mouthpiece, the extraoral part of which was glued air tight with Mastix to the upper and lower lip. A nose-clip was also used. From a thin metal tube in the mouthpiece a small part of the ventilatory air was continuously carried to an infra-red absorption analyzer for carbon dioxide (Capnograph, Godart) by means of suction.

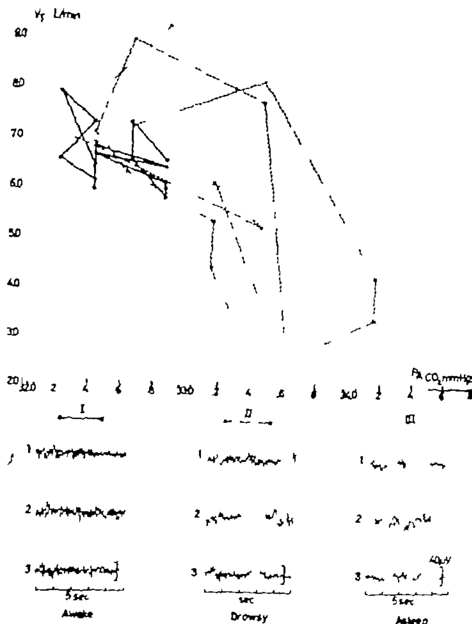


Fig. 1. Normal female subject, 26 years old. Diagram showing consecutive breath by breath analysis of ventilatory flow ($\dot{V}_T$) and alveolar "end-plateau" carbon dioxide tension ($P_A CO_2$). Three periods of each 77 sec were analysed when the subject was I awake at rest (lines drawn in full), II drowsy (dashed lines) and III asleep (dotted lines). (In the curve II four breaths were excluded in which the expiratory volume was too small to obtain an alveolar plateau.) Note small variations in $\dot{V}_T$ and $P_A CO_2$ when awake (I) and asleep (III), the latter group of values indicating lower ventilation and increased P_{CO_2}. Note further the scattered values pertaining to drowsiness (II).
The EEG samples were taken at the beginning (1), the middle (2) and at the end (3) of each period. In drowsiness the alpha periods in the EEG correlated to higher ventilation and lower carbon dioxide tension. The non-alpha periods with some theta and slow wave activity on the other hand, correlated to lower ventilation and higher $P_A CO_2$.



Fig. 2. Normal female subject, 20 years old. Diagram of consecutive breath by breath variations of ventilation and $P_{A\,CO_2}$ in relation to EEG during typical period of drowsiness with fluctuating awareness. The quotient $\dot{V}/P_{ACO_2}$ indicates "sensitivity" or ventilatory response to carbon dioxide. See text for further description.

The capnograph used permitted a detection of very small changes in P_{CO_2} (accuracy $\pm$ 0.3 mm Hg within the range 30 — 45 mm Hg) and reacted fast (90 % response time 0.1 sec). It was connected to writing unit (Omniscriptor Godart) with a linear frequency response up to 100 cycles per sec. The end-expiratory carbon dioxide tension (converted to STPD i. e. standard temperature and pressure of dry gas) for any single breath was measured from the end-plateau value of the record with an accuracy of $\pm$ 0.2 mm Hg. In normals at rest the end-expiratory P_{CO_2} may be considered as representative of the mean alveolar carbon dioxide tension (P_{ACO_2}) (COLLIER, AFFELDT and FARR 1955, GOFFERT and HEDDERICO 1956). The air sucked through the capnograph was shunted to the spirometer (Instrumenta) which was connected to the mouthpiece by two-way valve. A closed spirometric system with soda lime absorber and gas circulation fan was used. The added respiratory dead space of this apparatus was 15 ml.

During the whole recording period which generally lasted from two to four hours the oxygen consumed was continuously replaced through flow meter. The oxygen in the system was maintained at the level of 40 per cent in air. Thus, any stimulation from low arterial oxygen tension upon ventilation was avoided.

A continuous ink record was obtained from the spirometer. The ventilatory movements were also recorded on the same paper as the EEG by means of resistance variations in a potentiometer mounted on moving part of the spirometer. Thus, individual breaths and their relations to sudden changes in the EEG could easily be studied. From the spirographic records the ventilatory flow ($\dot{V}_T$ converted to BTPS, i. e. body temperature and atmospheric pressure of gas, saturated with water vapor) during single breaths was calculated.

Different states of wakefulness were classified from the EEG according to the system of LOOMIS, HARVEY and HOBART (1938), GIBBS and GIBBS (1951) and from JUNG (1953). As rule, the subjects were awake initially with a more or less continuous modulated alpha activity in the EEG (stage A). A period of drowsiness then followed with fluctuating awareness during which the alpha activity disappeared in the EEG and then developed some theta activity (stage B). In nine cases light sleep with slow waves and sleep spindles

Table I

Changes in ventilation (V_T) alveolar carbon dioxide tension ($P_{A\,CO_2}$) and quotient V_T per B (drowsy) and C (asleep) in five indi . . . selected at random.
The columns marked Index show the relative change in entilatory response to CO_2 as unit. All the values of V_T and P_{ACO_2} are the means of ten consecutive breaths (except . . . tage of wakefulness was changed.)

	Sex	Age	BSA m^2	A (Awake)				
				V_T l/min	$\frac{V_T}{BSA}$	P_{ACO_2} mm Hg	$\frac{V_{T/BSA}}{P_{ACO_2}}$	Index
1	F	28	1.73	5.40	3.09	32.4	0.95	1
2	F	22	1.70	4.95	2.91	32.3	0.90	1
3 a	F	25	1.98	5.16	2.61	37.2	0.70	1
b				5.00	2.53	37.9	0.67	1
4	M	49	2.00	8.15	4.08	32.3	1.26	1
5	F	60	1.53	4.46	2.92	36.5	0.80	1

(stage C) and in a few cases deeper sleep with high voltage delta activity (stages D and E) then followed. At the end of each recording period the sleeping subjects were usually aroused by being talked to and the ensuing changes were studied in the EEG and the pirogram.

In five of the subjects the quotient V_T/P_{ACO_2} (as the mean of ten consecutive breaths) was calculated for different states of wakefulness in order to get an index of the relative change in entilatory response to carbon dioxide between the different states of wakefulness.

Results

A complete series of recordings including wakefulness, drowsiness and sleep was obtained in 9 of the 16 subjects studied. In a few cases periodic breathing was recorded intermittently at a certain level of wakefulness. Such findings are not dealt with in the present report, which is concerned with those respiratory changes which in a more or less complete fashion could be recorded in all subjects which went to sleep and showed the characteristic EEG patterns.

The main finding of the present investigation, the close correlation between the EEG pattern and the ventilatory state was demonstrated both at steady states and during sudden or gradual changes in wakefulness.

At steady state, i.e. when the EEG pattern was homogenous for longer periods, the ventilation (V_T in l/min) and the alveolar carbon dioxide tension (P_{ACO_2} in mm Hg) varied very little. In diagrammatic form this fact is demonstrated in Fig. 1 in which three equally long periods during wakefulness, drowsiness and sleep were analyzed in one subject. It is seen that the V_T/P_{ACO_2} relationship in wakefulness and in sleep — with continuous alpha activity or

m² body surface area (BSA) / $P_{A_{CO_2}}$ during three stages of wakefulness, A (awake) ("CO_2 sensitivity") when the corresponding quotient V_T/BSA / $P_{A_{CO_2}}$ of stage A is taken for the values marked with where there were only fi consecutive breaths before the

B (Drowsy)				C (Asleep)			
V_T l/min	$P_{A_{CO_2}}$ mm Hg	$\frac{V_{T/BS}}{P_{A_{CO_2}}}$	Index	V_T l/min	$P_{A_{CO_2}}$ mm Hg	$\frac{V_{T/BS}}{P_{A_{CO_2}}}$	Index
4 68	33 6	0.79	0.83	4 18	33.2	0.72	0.73
3.72	35.2	0.62	0 69	3.58	35 8	0 59	0.63
4 67	36.7	0 64	0 91	—	—	—	—
4 52	36 8	0.62	0.92	—	—	—	—
.52	33 1	1 14	0.90	6.35	34.3	0 93	0.73
3 98	37.0	0.70	0 68	3.56	38 2	0 61	0.76

sleep-pattern in the EEG respectively — remained within narrow limits (Fig 1 circles and dots respectively) In contrast, the same relationship n drowsiness with its fluctuating EEG pattern showed large variations (Fig. 1 crosses)

In the diagram of Fig 1 it is also seen that during sleep the ventilation diminished and the $P_{A_{CO_2}}$ increased, i e. the points in the diagram shifted down to the right. This finding confirms fully the observations of MAGNUSSEN (1944) and the other authors mentioned above, who studied respiration in sleep.

In five subjects more complete data were obtained and the ventilatory response to carbon dioxide (the "carbon dioxide sensitivity" i.e. the $V_T/P_{A_{CO_2}}$ quotient) could then be compared during different periods of equal length showing EEG patterns A, B and C, representing wakefulness, drowsiness and sleep respectively In Table I it is seen that if the "sensitivity" during stage A was taken as unit, then "sensitivity" in stage B varied between 0.69 and 0 92 and n stage C between 0.65 and 0 76 In later experiments the "carbon dioxide sensitivity" was investigated more systematically by administering carbon dioxide in increasing doses (by rebreathing) at different levels of wakefulness. The results of these sensitivity tests will be the object of a later publication.

During changes from wakefulness to sleep, or the opposite, during arousal the ventilatory changes were also found to be very rapid In Fig 2 and 3 two examples are given of the concomitant EEG and respiratory changes when a subject went to sleep and was aroused.

In Fig 2 the rising $P_{A_{CO_2}}$ during successive alpha-loss and theta-slow wave appearance is clearly seen. The ventilation in this case showed an initial mo

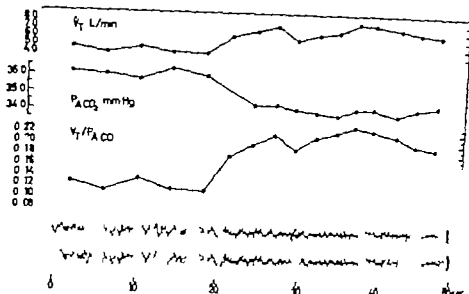


Fig 3 Normal female subject, 20 years old. Diagram of ventilation and P_{ACO_2} during arousal (t 20th second) See text.

sudden decrease. Such a lag in the P_{ACO} during the initial minute of sleep onset was often seen When, in this case, alpha activity appeared again for about 10 sec, there were simultaneous transient changes in ventilation and P_{ACO} Effects of arousal are shown in Fig 3

Both from Fig 1 and 2 it is evident that the state of drowsiness represents a non-steady state in which the ventilation varied very closely with the EEG changes between the wakefulness and the sleep type

Discussion

In the analysis of the results we have accepted the well established opinion (COLLIER et al 1955 ASMUSSEN and NIELSEN 1957 and others) that in normal humans at rest (with intact ventilation perfusion relationship and mechanics of the lung) there is a close parallelism between the arterial and the mean alveolar carbon dioxide tension It was also mentioned above that the expiratory end-plateau P_{CO_2} measured in normals at rest might be regarded as representative of the mean alveolar P_{CO} Hence, it would seem justified to conclude that under the present experimental conditions, i e. at rest in steady state, the alveolar end plateau P_{CO_2} recorded provides an index of changes in the P_{CO} of the arterial blood, which may affect the respiratory centers. This index should, however be considered a relative measurement since it cannot be concluded that the alveolo-arterial P_{CO} and the tension exerting its effects in the brain stem are identical

In the present breath by breath analyses the total ventilation, not the alveolar ventilation, has been related to the P_{ACO_2}. During regular breathing at rest the tidal volume varies closely in parallel with the effective alveolar volume (GRAY GRODINS and CARTER 1956, and others). Since, furthermore, the variations in ventilatory frequency at rest are small, the changes in total ventilation may be considered to vary closely in parallel with the effective alveolar ventilation. The ventilatory response of the respiratory centers to carbon dioxide may therefore be measured from the total ventilation at least when only moderate changes in ventilation are involved.

In view of the assumptions made in the aforegoing section we have interpreted the present results in the following way.

In general, the earlier findings of MAGNUSSEN (1944) and others have been confirmed. In sleep there is a decreased ventilatory response to carbon dioxide as compared to when awake. In addition, however, by the use of EEG it has been possible to show a close time relationship between transient, sudden, or gradual, shifts in wakefulness and changes in ventilation.

It should be emphasized, however, that the present technique has a limited time resolution since it is obviously limited to an intermittent breath by breath analysis. Hence there is no possibility from the present results to establish in detail whether the respiratory or the EEG changes occurred exactly simultaneously or whether one or the other was leading.

Although the present analysis does not include a second to second analysis of the ventilation — P_{ACO_2} — EEG relation, the time relations shown nevertheless seem evident enough. The marked general synchrony between ventilatory and EEG changes in the records which was seen in all subjects would seem to have a wider implication. Such a synchrony suggests, in fact, a close functional relationship between centers in the brain stem which regulate the respiration and those commonly considered to control th[e] cortical state of excitability as this is reflected in the EEG centers commonly referred to under the term the reticular activating system of the brain stem (MAGOUN 1958). A similar opinion has previously been expressed by BONVALLET, HUGELIN and DELL (1955) who studied experimentally the influence of carbon dioxide upon the reticular system of the brain stem.

The general conclusion reached above would imply that patients with some disturbance of their wakefulness regulation with ensuing EEG changes would also show respiratory irregularities. It is therefore of interest to note that SIEKER, HEYMAN and BIRCHFIELD (1960) reported depressed respiratory function with retention of carbon dioxide in eight cases of narcolepsy. Furthermore, POOLE (1960) recently reported a close correlation between changes in respiratory functions and the episodic EEG abnormalities which occurred in two cases of subacute encephalitis. In another series we studied respiration and EEG with the techniqu[e] described in a group of patients w[i]th heart neurosis and typical sigh breathing (Säufzeratmung) (i.e. patients with severe subj

heart symptoms without any demonstrable organic heart disease). In such cases there were also episodic transient subtle EEG changes which were correlated to the sighing breaths. The result of this investigation will be published later.

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Acta physiol. scand. 1961 51 239—246

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Distribution and Concentration of Adrenaline and Noradrenaline in the Adrenal Medulla of the Rat Following Reserpine-Induced Depletion

By

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Abstract

ERÄNKÖ, O and V HOPSU *Distribution and concentration of adrenaline and noradrenaline in the adrenal medulla of the rat following reserpine-induced depletion.* Acta physiol. scand. 1961 *51* 239—246. — Adrenaline and noradrenaline concentrations in the adrenal medulla were determined after chromatographic separation by fluorometry The chromaffin reaction, the iodate reaction and formalin-induced fluorescence were used as histochemical reactions and their intensities in the sections were quantitatively measured by microphotometry Four mg/kg of reserpine daily for 4 days caused a pronounced loss of adrenaline and noradrenaline and recovery took about 3 weeks. During the recovery adrenaline and noradrenaline concentrations increased proportionally The chemical and the histochemical measurements gave identical results. There was no evidence of local resynthesis of noradrenaline in excess of that of adrenaline. The results are t variance with observations reported earlier from other laboratories.

A large number of studies have been published demonstrating a pronounced loss of catecholamines from the adrenal medulla after administration of reserpine (CARLSSON and HILLARP 1956 ERÄNKÖ and HOPSU 1958 CAMANNI. LOSANA and MOLINATTI 1958, CALLINGHAM and MANN 1958 a, b, COUPLAND 1959 and others) The recovery of the medullary hormones after such depleti n has been much less studied we have found only the papers by CALLINGHAM and MANN (1958a, b) and COUPLAND (1959) to deal with t. CALLINGHAM MANN (1958a, b) found that the noradrenaline content of the drenals

a few days not only returned to normal but increased greatly above it, while the adrenaline content reached the control value much more slowly they studied only the content of catecholamines. COUPLAND (1959) used also histochemical reactions to investigate the distributions of adrenaline and nor adrenaline he reported that during the recovery phase noradrenaline is present in greater than normal amounts in those regions of the medulla which are normally concerned with storing adrenaline. Both CALLINGHAM and MANN (1958a) and COUPLAND (1959) concluded that methylation of noradrenaline is the rate-limiting factor in the synthesis of adrenaline.

In the present report observations are described in which both chemical and quantitative histochemical methods were used for studying the changes in the adrenomedullary catecholamines after reserpine administration.

Material and Methods

Experimental

Adult albino rats were used. In preliminary experiments, varying single doses of reserpine were injected and the depletion and recovery of the catecholamines was examined using only histochemical techniques (chromaffin reaction, iodate reaction, formalin-induced fluorescence). Similar experiments were then made using both histochemical and chemical methods of analysis.

The main experiments were carried out by using, first, 2 mg of reserpine per kg body weight subcutaneously daily for 3 consecutive days. This is twice the dosage used by CALLINGHAM and MANN (1958a) The daily dose was in later work increased to 4 mg/kg, and the rats were injected for 4 days. The animals were killed 1—24 days after the last injection by decapitation, together with bout the same number of similar but untreated control rats. The adrenals were thereupon removed and halved. One of the 4 halves was used for chemical determinations, the remaining 3 ones for histochemical studies

Chemical methods

The piec of adrenal was frozen on the microtome table and fresh sections were cut at 50 μ The sections were frozen-dried and pieces of pure medulla dissected out. These pieces were weighed to the nearest μg, the catecholamines were separated by paper hromat graph in a phenol-hydrochloric acid system and the separated amines were then determined fluorometrically The fluorometric method used was essentially that used earlier in this laboratory (ERÄNKÖ 1954) but higher concentration of ascorbic acid was used as proposed by EULER and FLODING (1955) This method is based on oxidation by ferricyanide t was first described by EHRLÉN.

Chromaffin reaction

Adrenal halves were immersed in mixture of 1 volume of 35 % formaldehyde and 19 volumes of 3 potassium dichromate for 24 hours and, subsequently in 3.5 formaldehyde f r ano ther 24 hours. Frozen sections were then cut t 50 μ these were mounted gl erol.

Iodate reaction

Adrenal halves were immersed in saturated solution of potassium iodate (HILLARP and HÖKFELT 1955) for 24 hours and, subsequently in 3.5 % formaldehyde for 24 hours. Sections cut at 50 μ were mounted in glycerol.

Formalin-induced fluorescence

Adrenal halves were fixed for 3—4 hours in solution containing 1 volume of 35 % formaldehyde, 6 volumes of 2 % calcium chloride and 3 volumes of distilled water. Sections cut at 50 μ were mounted in glycerol and studied for fluorescence.

Quantitative measurement of histochemical reactions

The optical densities of the chromaffin reaction and the iodate reaction were measured with microphotometer. Details of the apparatus and the method have been described elsewhere (ERÄNKÖ and RÄISÄNEN 1960). Measurements were carried out in two sections of each adrenal half, taking density readings from different parts of the medulla. In sections treated for the iodate reaction, the densities of the medullary background, consisting of the almost iodate-negative adrenaline-containing cells, and of the specific islets composed of the iodate-positive noradrenaline-containing cells were recorded separately. The density measurements were made against blank area on the slide, setting the photometer reading at zero density (100 % transmission). The results are expressed in terms of density (i. e. the negative logarithm of the transmission).

The fluorescence intensity was measured using photographic photometry (for details see ERÄNKÖ and RÄISÄNEN 1960). Readings were taken from both the weakly fluorescent medullary background and the fluorescent medullary islets, which correspond to the iodate-negative and -positive parts of the medulla, respectively. The intensity of the medullary fluorescence varied for methodological reasons somewhat from day to day (see ERÄNKÖ and RÄISÄNEN 1960); therefore, the fluorescence intensity was expressed in terms of per cent of the mean intensity of the corresponding region of the adrenal medulla of those control animals killed together with the injected animals.

Results

In the preliminary experiments with a single dose of reserpine, variable loss of medullary substances giving the three histochemical reactions was observed. With lower doses only some medullary cells lost their chromaffin reaction, while the normally iodate-positive and fluorescent medullary cell groups exhibited a total loss or a marked decrease in the intensity of these two histochemical reactions. This suggests in agreement with earlier observations (ERÄNKÖ and HOPSU 1958) that the effect was limited mainly to the noradrenaline-containing cells.

With larger single doses of reserpine and three daily injections of 2 mg/kg, the chromaffin reaction was negative or of decreased intensity in larger areas of the adrenal medulla, showing that the treatment had also caused a loss adrenaline. In all these experiments the medullary concentrations of

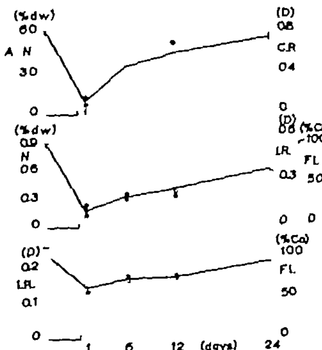


Fig. 1. Effect of reserpine on catecholamines of the adrenal medulla.

The injection period is indicated by white rectangle below each graph (4 mg/kg daily for 4 days). The time scale is days after the last injection.

Top graph: Concentration of both catecholamines (A + N, full circles), expressed as % of dry weight of medulla, and intensity of the chromaffin reaction (C. R.; open circles) expressed in terms of optical density. Each circle represents the mean of 6—8 animals. The line has been drawn between the mean chemical and histochemical values.

Center graph: Concentration of noradrenaline (N full circles) % of dry medullary weight; intensities of the iodate reaction (I. R. open circles) in terms of optical density and of the formalin-induced fluorescence (FL half-filled circles) of the corresponding mean control value. The intensities of the iodate reaction and the formalin-induced fluorescence were measured from the noradrenaline-containing cell islets.

Bottom graph: Intensities of the iodate reaction and the formalin-induced fluorescence in the medullary "background" adrenaline-storing cells. Symbols as in the center graph. Note that fluorescence is expressed as of the corresponding (background) mean control value therefore the fluorescence scales in this and the center graph are not comparable.

adrenaline and noradrenaline returned to normal within 4 days after the last injection. The chromaffin reaction, the iodate reaction and the intensity of the formalin-induced fluorescence were then similar of intensity and distribution as the corresponding reactions in the controls.

After daily injections of 4 mg/kg of reserpine during 4 consecutive days, pronounced effects were obtained. The results of the chemical analyses and of the quantitative measurements of the histochemical reactions are presented in Fig. 1. Each circle in each graph represents a mean value of a group of 6—8 animals, excepting the controls whose number was 26. The total number of animals in this experiment was 53. The injection period is indicated by a white rectangle below the base line. The scales of each graph have been adjusted so as to set the mean control values at the same height, which makes easier the comparison of the catecholamine concentrations and the intensities of the histochemical reactions.

The top diagram of Fig. 1 shows that there was an almost total loss of both

catecholamines 1 day after the last injection and that there was also a corresponding loss in the intensity of the chromaffin reaction, which actually was entirely negative in most sections, the residual loss of light being due to refraction rather than absorption (cf ERÄNKÖ and RÄISÄNEN 1960)

Both the catecholamine concentration and the intensity of the chromaffin reaction returned slowly to normal. When these variables were expressed as % of the mean control value, and these relative values were compared it was found that there was no statistically significant difference between the chemical and the histochemical data, although the mean density of the chromaffin reaction was somewhat higher 6 and 12 days after the last injection than the catecholamine concentration

The center diagram of Fig 1 shows corresponding changes in the noradrenaline concentration of the medulla and in the intensities of the iodate reaction and the formalin-induced fluorescence in the specific noradrenaline containing medullary cell islets. There is a surprisingly good agreement between these variables, and it is clearly evident that in this experiment no increase over the control value in the medullary concentration of noradrenaline occurred during the restitution period On the contrary noradrenaline concentration tended to return more slowly towards normal than the total catecholamine concentration.

The bottom diagram illustrates the changes in the intensities of the iodate reaction and of the formalin induced fluorescence in the medullary background, which consists of cells normally storing adrenaline. It is to be noted that the density and fluorescence scales are not the same as those in the center diagram, since again the mean control values have been placed at the same height from the zero line as in the two diagrams above.

No significant differences were found between the relative changes of these two reactions in the background both showing first a decrease and then a slow return to normal. The relative decrease was smaller in these histochemical reactions than in the noradrenaline concentration but this is to a great part due to the facts that scattering results in a non-specific loss of light even in practically colourless sections and that there was some residual non-specific fluorescence in the medullary cells even after practically total loss of noradrenaline. There was no increase in the intensity of these histochemical reactions over the mean control value at any stage of the experiment

Discussion

In our experiments 2 mg/kg of reserpine daily for three days caused a transient loss of catecholamines from the adrenal medulla but their concentration returned to normal within 4 days. Four consecutive daily injections of 4 mg/kg resulted in an almost complete loss of both adrenaline and noradrenaline, and recovery took several weeks.

In both experiments the concentrations of adrenaline and noradrenaline changed in parallel and in no instance was any absolute or relative increase above normal observed in the noradrenaline concentration. This is at variance with the results obtained by CALLINGHAM and MANN (1958a) and COUPLAND (1959). The former authors observed after 3 daily injections of 1mg/kg of reserpine first a decrease and after 3 days an increase in the noradrenaline content 300 % above the normal level. Adrenaline content also dropped first but it returned gradually to normal in the course of 2 weeks, during which time the temporarily elevated noradrenaline content slowly decreased to normal. In COUPLAND's (1959) experiment the noradrenaline content did not rise above the control level but it returned to normal before the adrenaline content; thus, the relative noradrenaline content temporarily increased. Moreover COUPLAND (1959) made calculations suggesting that the noradrenaline content of those cells of the adrenal medulla which normally store mainly adrenaline was increased during the recovery phase.

Several factors must be considered in trying to explain the differences in the results obtained by CALLINGHAM and MANN (1958a) by COUPLAND (1959) and by us. First of all the methods of catecholamine determination were different. CALLINGHAM and MANN (1958a) used rat's blood pressure and rat's uterus as bioassay techniques. These methods are subject to large errors when mixtures of adrenaline and noradrenaline are assayed (GADDUM and HOLZBAUER 1957). COUPLAND (1959) used a colorimetric method in which the differentiation of adrenaline and noradrenaline in a mixture is based on the fact that adrenaline is more readily oxidised at a low pH than noradrenaline. Also this method is subject to errors, particularly when much cortical extract, rich in ascorbic acid and a relatively small amount of noradrenaline are present in the mixture, as is the case in the adrenal medulla of the rat. In our work the amines were first separated by paper chromatography and thereafter an accurate fluorometric method of determination was used.

Fairly small errors in the estimation of the relative proportions of noradrenaline and adrenaline in COUPLAND's (1959) experiments would be sufficient to make non-significant the apparent small increase which he observed in the relative noradrenaline content during recovery. While COUPLAND's (1959) calculations seemed furthermore to suggest that there was an increase in the quantity of noradrenaline in the adrenaline-storing parts of the medulla, he did not histochemically observe any increase in the intensity of the iodate reaction in these areas. Even assuming that his figures for adrenaline and noradrenaline amounts were correct, the calculations which he made of the noradrenaline content of the medulla outside the specific cell islets are subject to criticism: they were based on measurements made by recording planimetrically the relative volume occupied by the noradrenaline storing cell islets in the medulla, which he found decreased during the recovery

Since the intensity of the iodate reaction was decreased and patchy in these cell islets during the recovery phase, COUPLAND (1959) himself found the delineation of the islets difficult and considered this in part responsible for the observed decrease in the volume of the islets after reserpine administration. Therefore, the apparently lower volume of the cell islets may indicate a temporary loss of noradrenaline from the noradrenaline-storing cells in the islets, rather than a real diminution in the size of the cell islets and an increase of noradrenaline concentration in the adrenaline-storing medullary cells outside the islets.

The observations made by COUPLAND (1959) and those of the present study are in agreement concerning the following points reserpine caused in both studies a loss of both adrenaline and noradrenaline as well as a decrease in the intensities of the chromaffin and of the iodate reaction, and during the recovery phase there was no increase of the absolute noradrenaline content bove the normal level. In both studies, furthermore, the intensity of the iodate reaction in the medullary background, i. e in the cells normally storing adrenaline, did never increase above normal indeed, our quantitative measurements indicated a decrease in this variable.

While COUPLAND's work and our differ therefore, mainly in the interpretation, the results obtained by CALLINGHAM and MANN (1958a) are entirely different from those of COUPLAND and ours as far as changes in the noradrenaline content are concerned. It is difficult to find a pertinent explanation for this difference.

It does not seem likely that the very great increase which CALLINGHAM and MANN observed in the pressor response obtained with the extracts made from adrenals in the recovery phase could have been caused by errors in the assay However their evidence of the increase in the noradrenaline content is based on the assumption that the increase in the pressor response is due mainly to this amine. It is, however not altogether impossibl that the high assay values are in fact due to some other pressor substance whose concentration increases in the medulla d ring recovery

Another possibility is that differences in the susceptibility of the rats towards reserpine in the different laboratories are of importance. Strain-dependent differences in th catecholamin loss after reserpine administration have earlier been demonstrated by COUPLAND (1958) who observed that Wistar rats required three times as much reserpine for an equal depletion as rats of the Sprague Dawley strain. Dietary differences may also play rol in this respect

Further studies are in progress on this problem in collaboration with Dr K. R. BUTTERWORTH who belongs to the same research group as Dr MANN and Dr CALLINGHAM.

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Distribution and Concentration of Adrenaline and Noradrenaline in the Adrenal Medulla of the Rat Following Depletion Induced by Muscular Work

By

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Abstract

ERÄNKÖ, O and M. HÄRKÖNEN *Distribution and concentration of adrenaline and noradrenaline in the adrenal medulla of the rat following depletion induced by muscular work.* Acta physiol. scand. 1961 *51* 247—253. — The rats were allowed to run for 16 hours, to rest for 12 hours and t run again for 10 hours. They were killed immediately 3, 6, 12 or 19 days thereafter Concentrations of adrenaline and noradrenaline in the adrenal medulla were measured chemically The intensities of the chromaffin reaction, the iodate reaction and formalin-induced fluorescence in adrenal sections were quantitated by microphotometry Running caused an almost complete loss of both catecholamines from the adrenal medulla and the histochemical reactions turned almost negative. The catecholamine concentrations and the histochemical reactions returned to normal in 6 days. During the recovery phase the behaviour of the noradrenaline-storing medullary cells differed histochemically from that of the adrenaline-storing cells.

Muscular work is known to be potent stimulator of the adrenal medulla (g EULER 1956, KÄRKI 1956) However we are n t aware of studies in which the concentrations in the adrenal medulla of both adrenaline and noradrenaline had been tudied fter long-lasting muscular work. HÖKFELT (1951 reported that after swimming for 30—90 min th noradrenaline content of rat adrenals showed a tendency to rise, whereas the adrenaline content

tended to decline. He did not study the effect of longer lasting work. Since interesting features in the recovery of the catecholamines have been reported after depletion by pharmacological substances, e g reserpine (CALLINGHAM and MANN 1958 a, b COUPLAND 1959 ERÄNKÖ and HOPSU 1961) we undertook a similar study using muscular work as a stimulus.

Material and Methods

Experimental

Adult albino rats were used. They were allowed to run in a rotating wire cylinder for varying periods. In preliminary experiments the effect on the adrenal medulla was investigated histochemical methods only using the chromaffin reaction, the iodate reaction and formalin-induced fluorescence (see below) as indicators of the amount of the catecholamines in the medulla. In these experiments, a single period of work lasting up to several hours resulted only in a partial depletion of the medullary catecholamines, and rapid restoration to normal occurred within a day after the work had been stopped.

However when the rats were allowed to run for 16 hours, to rest thereafter for 12 hours and to run again for 10 hours, an almost total disappearance of catecholamines was observed, as indicated by the three histochemical reactions, which all turned almost negative. This experimental arrangement, which proved reproducible was then adopted as a routine procedure and the results to be reported in this paper have been obtained by using it.

The animals were killed by decapitation immediately 3, 6, 12 or 19 da[illegible] after the end of the second running period, together with about the same [illegible]umber of similar but untreated control rats. Both adrenals were cut into two pieces of equal size One piece was used for chemical analysis, the remaining 3 pieces for histochemical [illegible]tud[illegible]es.

Chemical methods

Fresh adrenal sections cut at 50 μ were freeze-dried and pieces of pure dr[illegible] medulla were dissected out and weighed. The catecholamines were either first separated by chromatography eluted and determined separately by fluorometry after oxid[illegible]tion [illegible]t pH 6 (e g EULER and FLODING 1955) or determined directly in the extract making the fluorometric measurements at two exciting wavelengths, 366 mμ and 436 mμ for the determination of the relative proportion of noradrenaline in the mixture (g COHEN and GOLDENBERG 1957) For further details of the method, see ER[illegible]NKÖ and RÄIHÄNEN (1960) Since the two methods ga[illegible]e comparable results, the data obtained with them were pooled and handled together

Histochemical methods

One of the three remaining adrenal pieces was immersed in [illegible] mixture of potassium dichromate and formalin for the demonstration of both catecholamines by the chromaffin reaction, another was plunged into a saturated solution of potassium iodate for the demonstration of noradrenaline (HILLARP and HÖKFELT 1955) and the third one was fixed in a mixture of calcium chloride and formalin [illegible] develop the formalin-dependent fluorescence in the noradrenaline cell islets. Frozen sections cut at 50 μ were mounted in glycerol. For details see the previous paper (ERÄNKÖ and HOPSU 1961)

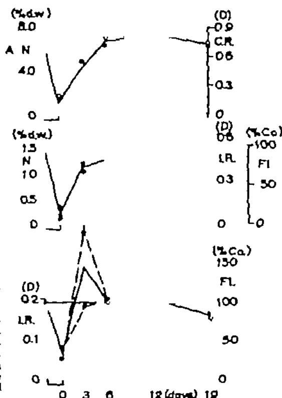


Fig. 1. Effect of muscular work on catecholamines of the adrenal medulla.

The running period is indicated by white rectangle below the base line. Each circle represents the mean of group of 5—7 animals. The solid line has been drawn between the mean chemical and histochemical values.

Top graph. Concentration of both catecholamines (A + N full circles), % of dry medullary weight, and optical density (D) of the chromaffin reaction (C. R.; open circles).

Center graph. Concentration of noradrenaline (N full circles), % of dry medullary weight, optical density (D) of the iodate reaction (I. R., half-filled circles) and intensity of the formalin-induced fluorescence (Fl open circles), % of the corresponding mean control value. The intensities of the iodate reaction and the fluorescence were measured from the noradrenaline-storing cell islets.

Bottom graph. Iodate reaction and formalin-induced fluorescence in the medullary "background" adrenaline-storing cells. Note that fluorescence is expressed as of the corresponding (e. background) mean control value. Therefore the absolute values of fluorescence in this and the center graph are not comparable

Quantitative measurement of histochemical reactions

The optical densities of the chromaffin reaction and the iodate reaction, as well as the intensity of the formalin-induced fluorescence were measured by microphotometry. The method has been described in detail elsewhere (ERÄNKÖ and RÄISÄNEN 1966, see also ERÄNKÖ and HOPSU 1961). Measurements of the intensities of the iodate reaction and the formalin-induced fluorescence were carried out separately on the noradrenaline-containing cell islets and on the medullary background consisting of cells normally storing mainly adrenaline.

Measurement of medullary volume

A separate experiment was carried out to study the effect of running on the volume of the adrenal medulla. The animals were killed immediately, 3 or 6 days after running in two consecutive days according to the schedule described earlier in this paper. One adrenal was immersed in the dichromate-formalin mixture, the other in saturated iodate solution. After 24 hours, both glands were postfixed in 4 formaldehyde. The medullary volume was planimetrically measured from a complete series of sections cut from the gland fixed in dichromate-formalin (ERÄNKÖ 1954

Results

The results are presented in Fig 1 Each circle represents a mean of a group consisting of 5—7 rats, excepting the controls, whose number was 28. The total number of animals in this experiment was 58. The running period is indicated by a white rectangle below the base line. The scales of each graph have been adjusted by setting the mean control values at the same height. The fluorescence intensity is expressed as percent of the corresponding mean control value.

The top diagram indicates a pronounced loss of catecholamines and a decrease in the intensity of the chromaffin reaction immediately after running Both variables returned to normal in 6 days, and remained at the normal level thereafter The changes in the catecholamine content and in the chromaffin reaction were parallel, and in no stage of the experiment was there any significant difference between their mean values.

The center diagram illustrates the changes in the noradrenaline concentration of the medulla and in the intensities of the iodate reaction and of the formalin-induced fluorescence in the specific cell islets normally storing noradrenaline. The changes in these variables are closely associated showing a pronounced loss of noradrenaline after running and a return to normal in a few days. Comparison of this diagram and the upper one seemingly suggests that the noradrenaline concentration was nearer the normal value three days after the running than was the total catecholamine concentration which would indicate that the rate of formation of noradrenaline was higher than that of adrenaline. However there was no statistically significant difference on the third day between the mean of the total catecholamine content expressed as percent of the control mean and that of the mean noradrenaline content expressed in the same way Although the means of the three variables in the center diagram remained below the corresponding normal mean on the 6th, 12th and 19th day the differences from the corresponding control means were not statistically significant.

The bottom diagram illustrates changes in the intensities of the iodate reaction and of the formalin induced fluorescence in the medullary background, i.e., in the cells normally storing adrenaline Because the scales have been adjusted by setting the mean control value, which is much smaller than in the cell islets, at the same height as in the two upper graphs, smaller absolute changes correspond to relative changes equal to those in the upper graphs.

Running caused a decrease in the intensities of the iodate reaction and the formalin-induced fluorescence in the background, and the values returned quickly towards normal. On the third day the intensity of the iodate reaction was already at the control level This is of interest because neither the noradrenaline concentration in the medulla nor the histochemical reactions in the noradrenaline cell islets had returned to normal during the same time.

Table I. Effect of running on the volume of the adrenal medulla

Treatment	Number of animals	Volume of medulla (per cent of controls)		P*
		Mean	SD	
Controls	8	100.0	9.6	–
Running, killed immediately	9	126.4	19.1	~ 0.003
Running, killed after 3 days	7	108.5	6.5	> 0.05

*The value of P has been calculated with the aid of the t-test and shows the significance of the difference from the control group.

The difference of the means of the intensity of the iodate reaction in the specific cell islets and that in the background, both expressed in terms of percent of the corresponding control mean, is statistically significant ($P \sim 0.02$).

The most striking feature in this graph is, however, the very high fluorescence intensity of the background on the 3rd day. This increase is statistically highly significant ($P < 0.001$). From the 6th day on the intensity of both the iodate reaction and the fluorescence kept near the corresponding control mean and did not differ from it significantly.

Table I shows the changes in the volume of the adrenal medulla immediately and 3 days after the last running period. Running caused a 26 percent increase in the medullary volume but after 3 days the mean volume did not any more differ significantly from that of the control group. The chromaffin reaction and the iodate reaction were almost negative immediately after running and their intensities were according to visual judgement about normal after 3 days rest. However, after 3 days the intensity of the iodate reaction in the background of the medulla tended to be higher than in the controls.

Discussion

Long-lasting muscular work caused in the present study a pronounced depletion of both adrenaline and noradrenaline from the adrenal medulla. It is not possible to say in how far this depletion was due to muscular work alone and how much other factors such as general exhaustion, lack of sleep and diminished intake of food and water contributed to the effect observed. Fasting or lack of water for several days cause hardly any changes in the intensity of the chromaffin reaction in the adrenal medulla of the rat (ERÄNKÖ unpublished), but they both may nevertheless potentiate the effect of muscular work. However, whatever the mechanism of the depletion is, it was reproducible and reversible.

The concentrations of both catecholamines and the intensities of the histochemical reactions employed returned to normal in 6 days. This period is much shorter than that required for recovery after reserpine administration, which resulted in a comparable initial loss of catecholamines from the adrenal medulla (*cf.* Fig. 1 in this paper with Fig. 1 in ERÄNKÖ and HOPSU 1961). Such a difference suggests that reserpine seriously interferes with the synthesis and/or storage of adrenaline and noradrenaline in the adrenal medulla, while even prolonged muscular work has less or no such effect. This fits well in with the recent observations demonstrating that reserpine prevents the storage of catecholamines in the cytoplasmic granules (BERTLER, HILLARP and ROSENGREN 1960).

In general, the results of the chemical determinations agreed in the present study well with those obtained by quantitating the histochemical reactions. Likewise, the changes in the adrenaline and noradrenaline concentrations were parallel. In these respects the results were similar as those obtained in the reserpine experiment (ERÄNKÖ and HOPSU 1961).

However, 3 days after the end of the running period, *i.e.* when an active *resynthesis of the depleted amines took place, an interesting divergence was* observed between the intensities of the iodate reaction and the formalin-induced fluorescence in the medullary background: while the intensity of the iodate reaction was equal to that in normal controls, the intensity of the fluorescence was almost twice the normal value.

This shows that the formalin-induced fluorescence, although it usually is parallel with the iodate reaction, is not always so. For this there are three possible explanations. First, the formalin-induced fluorescence may not, like the iodate reaction, depend, at least exclusively, on noradrenaline but to some other substance whose concentration tends usually but not always to vary parallelly with that of noradrenaline. Second, the increase in the intensity of the fluorescence is perhaps not due to an increase in the amount of the fluorescent substance but may be caused by some other change, *e.g.* by a loss of a substance quenching the fluorescence: this explanation is compatible with the assumption that noradrenaline is responsible for the fluorescence. Third, the iodate reaction may not demonstrate noradrenaline outside the granules.

Of these alternatives the first one appears most likely but the nature of the postulated fluorescent substance, if other than noradrenaline, remains obscure. At any rate, the observation completes two earlier ones in which a discrepancy was observed between the intensities of the fluorescence and the iodate reaction (ERÄNKÖ 1955, ERÄNKÖ, HOPSU and RÄISÄNEN 1959).

On the 3rd day, the intensity of the iodate reaction in the medullary background was already normal although the total catecholamine concentration and the noradrenaline concentration were still below normal. Assuming that the iodate reaction is a faithful indicator of the local noradrenaline concentra-

tion, this means that the rate of formation of noradrenaline in the normally adrenaline-storing cells was higher than the rate of the subsequent methylation to noradrenaline. It is noteworthy that CALLINGHAM and MANN (1958 a, b) and COUPLAND (1959) arrived at the same conclusion in experiments in which the loss of catecholamines was induced by reserpine. However since the results obtained in this laboratory with reserpine (ERÄNKÖ and HOPSU 1961) are different and since the matter is under investigation in collaboration with the London laboratory it seems better to postpone the discussion of this matter until the results of the joint study are available.

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The Effect of Testosterone, Progesterone and Estrogen on the Acetylating Activity in Rat Liver and Kidney Preparations

By

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Abstract

Luukkainen, T. *The effect of testosterone, progesterone and estrogen on the acetylating activity in rat liver and kidney.* Acta physiol. scand. 1961. 51. 254—262. — The acetylation of *p*-aminobenzoic acid has been studied *in vitro* in rat liver and kidney preparations after the treatment of the rats with gonadal steroids. It was observed that none of these steroids in the doses used had any effect on the acetylation in the liver tissue. In the kidney tissue of male rats estrogen treatment clearly decreased the acetylation, progesterone had no effect, and testosterone may increase the acetylating kidney tissue in female rats.

The capacity of male and female rats to acetylate aromatic amines has been found to be different. Reports of Dunn and Ralli (1951), Franz and Lata (1953, 1957) and Fishkin and Lata (1957, 1958) have indicated that male rats *in vivo* have a greater acetylating activity than female rats. It has also been demonstrated that sex hormones administered to rats are capable of changing their acetylation power *in vivo* (Fishkin and Lata 1958).

The sex-linked difference in the acetylation was found in this laboratory to exist also *in vitro*, where some of the disturbing factors of the *in vivo* experiments can be eliminated (Luukkainen 1958). The effect of sex glands on acetylation was clearest in the kidney tissue. The kidney preparations of the male rats acetylated larger amounts of *p*-aminobenzoic acid than those of the female rats. Castration of th male rats reduced the acetylating activity, whereas ovariectomy increased the acetylation in the kidney preparations.

This communication deals with the effects of gonadal steroid treatment on the *in vitro* acetylation of the rat liver and kidney tissue.

Materials and Methods

The rats used were of the Wistar strain. The rats for the experiments were selected by weight from healthy animals of the same age. The animals were first tested *in vivo* with *p*-aminobenzoic acid (PAB) to find out their acetylating power. In the *in vivo* tests the rats were given 15 µmoles of PAB through a stomach tube in 5 ml of water. Urine was then collected during an experimental period of 4 hours and the urine samples were analyzed to determine the acetylation percentage and amount of amine excreted. On the basis of these tests the males with the highest and the females with the lowest acetylation power were castrated to make the biological variation between the experimental animals as small as possible.

One month later the animals were tested again *in vivo* with PAB. According to the results of this test each of the four groups of animals, *i. e.*, the unoperated as well as the castrated male and female rats, were divided into control and experimental groups having a nearly equal acetylation ability.

The steroid preparations used were as follows

Testosterone — Testosterone propionate and testosterone enanthate (Primoteston and Primoteston depot, Schering AG Berlin)

Progesterone — Progesterone and 17 α-hydroxyprogesterone caproate (Primolut and Primolut depot, Schering AG Berlin)

Estrogen — Estradiol benzoate (Primogyn B oleosum, Schering AG Berlin)

The hormones were diluted with their original solvent oils to the desired concentrations and given intramuscularly to the experimental rats in 0.1 ml portions. The control animals were treated with the same volume of the corresponding solvent oil.

The effect of the treatment was followed by subjecting the rats to the *in vivo* acetylation tests.

For the *in vitro* experiments the animals were fasted for one night and killed by decapitation. The viscera were removed and quickly placed on ice. From each rat two 2 g liver samples and both kidneys were weighed and used for the experiments. The tissues were dropped into incubation flasks containing 10 ml of ice cold incubation medium of the following composition:

Physiological sodium chloride solution	200 ml
0.154 M potassium chloride solution	8 ml
1.0 M sodium acetate solution	8 ml
0.02 M neutralized *p*-aminobenzoic acid	4 ml
0.1 M potassium sodium phosphate buffer pH 7.4 to make	400 ml

The volume of medium used thus contained 2 µmoles of PAB and 200 µmoles of acetate for 2 g of liver tissue or for one kidney.

The tissue was minced in the incubation flasks with scissors into small sections as described previously (Luukkainen 1958). Then the flasks were gassed for 10 minutes with oxygen and after this procedure incubated in a water-bath at 37 C with constant shaking. After incubation, duplicate samples of 1 ml were taken from each flask and transferred to centrifuge tubes containing 4 ml of 5 per cent trichloroacetic acid for deproteinization. The determination of the *p*-aminobenzoic acid conjugates was made by the method of Bratton and Marshall (1939) calculating the amount of acetylated conjugates from values obtained without hydrolysis and after hydrolysis.

I wish to express my gratitude to Messrs. Schering AG for kindly supplying the steroids.

Results

There was no observable difference between rats treated with testosterone and control rats when they were tested *in vivo* with *p*-aminobenzoic acid twice a month during the treatment. The dose of testosterone used was 500 μg of testosterone propionate i. m. per day for 28 days, then 3.5 mg of testosterone enanthate i. m. weekly for the following 6 weeks, and testosterone propionate 500 μg per day i. m. for the remaining 20 days. The dose was thus 500 μg per day during 90 days. This dose had no effect on the acetylating capacity of liver *in vitro* as shown in Table I

Table I Acetylation of p-aminobenzoic acid (PAB) in liver after testosterone treatment

Each incubation flask contained 2 g of liver sections, 2 μmoles of PAB and 200 μmoles of acetate in 10 ml of medium, pH 7.4 Oxygen as gas phase and incubation time 4 hours. Testosterone 500 μg i. m. per day for 90 days before experiment.

Sex of rats	Treatment	Number of rats	Acetyl-PAB formed, μmoles	S. D.	S. E.	Significance of change from controls
Females	Testosterone	5	0.72	0.015	0.007	None
	Control	4	0.77	0.147	0.073	($P > 0.05$)
Castrated females	Testosterone	6	0.62	0.094	0.012	None
	Control	5	0.68	0.093	0.041	
Males	Testosterone	4	0.67	0.131	0.066	None
	Control	5	*0.73*	*0.069*	*0.031*	
Castrated males	Testosterone	6	0.72	0.067	0.027	None
	Control	5	0.71	0.060	0.027	

In the kidney as seen from Table II the amount of acetylated *p*-aminobenzoic acid formed per gram of kidney tissue is smaller in testosterone treated castrated male rats than in corresponding control rats. However there is the typical testosterone effect (KORENCHEVSKY and DENNISON 1934 KORENCHEVSKY *et al* 1937 (a) and 1937 (b) *i. e.* increasing weights of kidneys, as stated in Table III This effect naturally influences only the results of the treated animals. For comparison with the *in vivo* experiments reported in the literature, Table II also shows the acetylated conjugates formed per whole kidney As in the *in vivo* experiments the change in weight of the kidneys then remains unaccounted for The kidneys of ovariectomized treated female rats and perhaps of unoperated treated female rats produce more acetylated conjugates than the corresponding controls when this manner of calculation is used. The total acetylating capacity of the kidney is the same in the treated and control groups of male and castrated male rats.

Table II Acetylation of p-aminobenzoic acid (PAB) in kidney after testosterone treatment

One kidney as tissue sample other experimental conditions as in Table I

Sex of rats	Treatment	Acetyl-PAB formed per 1 g of kidney µmoles	S. D	S. E.	Significance	Acetyl-PAB formed per kidney µmoles	Significance of chang from controls
Females	Testosterone	0.57	0.089	0.040	None	0.55	+ P < 0.02
	Control	0.45	0.072	0.036		0.33	
Castrated females	Testosterone	0.64	0.075	0.031	None	0.69	+ P < 0.01
	Control	0.55	0.091	0.040		0.50	
Males	Testosterone	0.65	0.131	0.066	None	0.71	None
	Control	0.90	0.068	0.031		0.91	
Castrated males	Testosterone	0.65	0.095	0.039	−P<0.05	0.80	None
	Control	0.82	0.051	0.023		0.73	

Table III Body and organ weights of testosterone treated rats

Sex of rats	Treatment	Mean of body weights, g	Mean of liver weights, g	Mean of kidney weights, g
Females	Testosterone	187	7.490	0.960
	Control	170	7.560	0.740
Castrated females	Testosterone	230	8.760	1.080
	Control	245	9.250	0.910
Males	Testosterone	214	8.270	1.090
	Control	268	9.680	1.010
Castrated males	Testosterone	251	9.590	1.220
	Control	238	8.610	0.890

The dose of progesterone was 500 µg per day i. m. for 28 days, followed by 6 weeks administration of 3.5 mg i. m. hydroxyprogesterone caproate weekly and after this again 500 µg of progesterone per day for 20 days.

The results of the experiments with liver tissue are presented in Table IV. There are no statistically significant differences in the acetylation between treated and control rats. The average amount of formed acetylated p-aminobenzoic acid conjugates in the progesterone treated male rats is slightly lower than in the untreated rats, probably reflecting only slight hormonal castration caused by progesterone.

Table IV Acetylation of p-aminobenzoic acid (PAB) in liver after progesterone treatment

Each incubation flask contained 2 g of liver sections, 2 μmoles of PAB and 200 μmoles of acetate in 10 ml of medium, pH 7.4 Oxygen as gas phase and incubation time 4 hours. Progesterone 500 μg i. m. per day for 90 days before experiment.

Sex of rats	Treatment	Number of rats	Acetyl-PAB formed, μmoles	S. D	S. E.	Significance of change from controls
Females	Progesterone	10	0.60	0.09	0.028	None
	Control	9	0.65	0.09	0.030	($P > 0.05$)
Castrated females	Progesterone	9	0.65	0.38	0.126	None
	Control	9	0.64	0.38	0.126	
Males	Progesterone	10	0.70	0.44	0.140	None
	Control	9	0.87	0.09	0.030	
Castrated males	Progesterone	8	0.63	0.47	0.170	None
	Control	9	0.59	0.07	0.025	

On examination of the mean values of the acetylated conjugates formed in the kidney preparations of progesterone treated and control rats it is observed that there are no significant differences between these two groups (Table V) Table VI shows the mean weight of body liver and kidney of rats in the progesterone series. There is no kidney enlargement, as after testosterone treatment (Table III)

Table V Acetylation of p-aminobenzoic acid (PAB) in kidney after progesterone treatment

Each incubation flask contained one kidney in sections, other experimental conditions as in Table IV

Sex of rats	Treatment	Acetyl-PAB formed per 1 g of kidney μmoles	S. D.	S. E.	Significance of change from controls
Females	Progesterone	0.36	0.01	0.003	None
	Control	0.36	0.03	0.016	($P > 0.05$)
Castrated females	Progesterone	0.40	0.06	0.019	None
	Control	0.49	0.08	0.025	
Males	Progesterone	0.72	0.36	0.115	None
	Control	0.79	0.06	0.020	
Castrated males	Progesterone	0.56	0.06	0.022	None
	Control	0.59	0.08	0.027	

Table VI Body and organ weights of progesterone treated rats

Sex of rats	Treatment	Mean of body weights, g	Mean of liver weights, g	Mean of kidney weights, g
Females	Progesterone	179	7.720	0.760
	Control	169	7.150	0.760
Castrated females	Progesterone	217	9.320	0.890
	Control	225	9.130	0.890
Males	Progesterone	281	11.060	1.060
	Control	297	11.210	1.160
Castrated males	Progesterone	262	10.730	0.990
	Control	283	9.580	1.070

When the effect of estrogen was studied estradiol benzoate was given 2 µg per day i. m. for 120 days. The duration of the treatment was so long because in the *in vivo* tests there were some rather insignificant decreases in the acetylation of male rats after 90 days. This decrease did not come out more clearly by waiting the following 30 days. The results of the experiments with liver tissue are presented in Table VII. We see that there are no differences in the acetylation after the estrogen treatment between th treated and control rats.

Table VII Acetylation of p-aminobenzoic acid (PAB) in liver after estrogen treatment

Each incubation flask contained 2 g of liver sections, 2 µmoles of PAB and 200 µmoles of acetate in 10 ml of medium, pH 7.4. Oxygen as gas phase and incubation time 4 hours. Estradiol benzoate 2 µg i. m. per day for 90 days before experiment.

Sex of rats	Treatment	Number of rats	Acetyl-PAB formed, µmoles	S. D.	S. E.	Significance of change from controls
Females	Estradiol	5	0.79	0.11	0.019	None
	Control	5	0.77	0.08	0.029	(P 0.05)
Castrated females	Estradiol	6	0.80	0.07	0.027	None
	Control	5	0.81	0.16	0.071	
Males	Estradiol	5	0.71	0.14	0.061	None
	Control	5	0.74	0.11	0.018	
Castrated males	Estradiol	7	0.79	0.10	0.016	None
	Control	5	0.70	0.06	0.025	

The same small dose of estradiol, which in the liver has no effect, clearly decreases the acetylating ability of the kidney. This decreasing effect of estrogen comes out already in the examination of the average amounts of acetylated conjugates in μmoles (Table VIII). The numerical values of formed acetyl-PAB are smaller in all the treated animals than in the controls. The effect is, however, statistically significant only in both male rat groups. As seen from Table IX, this decrease is not an indication of change in the weight of the kidneys.

Table VIII. Acetylation of p-aminobenzoic acid (PAB) in kidney after estrogen treatment

Each incubation flask contained one kidney in sections, other experimental conditions as in Table VII.

Sex of rats	Treatment	Acetyl-PAB formed per 1 g of kidney μmoles	S. D.	S. E.	Significance of change from controls
Females	Estradiol	0.25	0.01	0.007	None
	Control	0.31	0.07	0.029	
Castrated females	Estradiol	0.31	0.07	0.029	None
	Control	0.42	0.01	0.006	
Males	Estradiol	0.31	0.02	0.007	– $P < 0.01$
	Control	0.57	0.07	0.032	
Castrated males	Estradiol	0.30	0.01	0.003	– $P < 0.001$
	Control	0.58	0.06	0.025	

Table IX. Body and organ weights of estrogen treated rats

Sex of rats	Treatment	Mean of body weights, g	Mean of liver weights, g	Mean of kidney weights, g
Females	Estradiol	197	8.100	0.930
	Control	215	8.590	0.930
Castrated females	Estradiol	202	9.320	0.920
	Control	237	8.080	0.880
Males	Estradiol	248	9.370	0.940
	Control	271	10.060	1.060
Castrated males	Estradiol	249	10.040	0.980
	Control	263	8.890	0.880

Discussion

The ability of female rats to acetylate arylamine has been shown by FISHKIN and LATA (1957 1958) to be increased after testosterone treatment in vivo In the present investigation no differences were found between the experimental and control animals in the acetylation of liver tissue after testosterone treatment. Testosterone increased the weight of the kidneys, as shown earlier by KORENCHEVSKY and DENNISON (1934) and KORENCHEVSKY et al (1937 a, 1937 b) This increase in weight is an indication that the dose of steroid used has had an effect. However there is no effect on the acetylation when calculated per gram of kidney It has to be borne in mind however that the total acetylating capacity of the kidneys increases in the treated female and ovariectomized groups. This finding corresponds to the above mentioned in vivo results. In vivo the effects of the kidney enlargement cannot be observed.

In the progesteron group there is no difference between treated and untreated rats in the acetylation in either tissues tested. This observation supports the idea that progesterone alone has no influence on the sex-linked difference found in arylamine acetylation.

Estrogen treatment has, according to the presented results, no effect on liver acetylation Estrogen acts in a different manner in the kidney where it is a potent depressor of acetylation even at a relatively low daily dose (2 μg) of estradiol. The inhibition of acetylation is in agreement with previous findings, where ovariectomy elevated the level of the acetylation (LUUKKAINEN 1958) The observed decrease in acetylation does not seem to be due to activation of a deacetylating enzyme in the used tissue preparations, where no deacetylation of the acetylated p-aminobenzoic acid was found (KOIVUSALO and LUUKKAINEN 1959)

The different sensitivity of acetylation to estrogen in the kidney and liver are presumably based on a difference in the conjugation system in these two organs and in the kidney it may have some correlation with the sex-dependent differences in structure. Morphological structure differences have been found to exist in the kidney between the two sexes (CRABTREE 1940 ESCHENBRENNER and MILLER 1945) The role of the kidney in the acetylation difference between male and female rats, also in vivo might be of some importance

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The Urinary Excretion of Galactose and its Significance in Clinical Intravenous Galactose Tolerance Tests

By

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Abstract

TYGSTRUP, N. *The urinary excretion of galactose and its significance in clinical intravenous galactose tolerance tests.* Acta physiol. scand. 1961. 51. 263—274. — The disappearance of galactose from the blood after intravenous administration may be used for measurement of the liver function, if correction for the urinary loss of galactose is made. The present study was performed in order to determine the significance of this loss. It was found that urinary excretion of galactose was unimportant in relation to hepatic elimination at plasma concentrations below about 500 mg/l (approximately 3 per cent of the total elimination) but at higher concentrations the ratio between urinary excretion and hepatic elimination increased, as the former rises with the concentration, and the latter remains constant. After single injection of about 500 mg/kg body weight on an average 10 per cent of this amount was excreted in normal subjects as well as in patients with cirrhosis. This percentage could be used for practical calculation of the contribution of the urinary loss to the total elimination rate of galactose during the interval in which the latter remains essentially constant. The experiments indicate a low renal threshold for galactose, but no evidence for reabsorption T_m was found within the range of plasma concentrations studied (up to 3 000 mg/l). The renal clearance of galactose was significantly decreased in patients with cirrhosis. The implications of these findings for the calculation of the maximal hepatic galactose elimination rate from the plasma concentration curve following single injection are examined in an appendix.

The fact that the plasma galactose concentration-time curve following a single intravenous injection of galactose is essentially rectilinear over a considerable range of plasma concentrations (TYGSTRUP and WINKLER 1954) indicates that in this range the elimination *of galactose mainly depends on processes* which are saturated at such concentrations. As galactose is predominantly eliminated by the liver the maximal galactose elimination rate is determined largely by the metabolic *status of the liver*.

It is well known however that some extrahepatic elimination of galactose does occur (BOLLMAN, MANN and POWER 1935) chiefly by way of renal excretion. If urinary loss of galactose is significant under the conditions used in clinical intravenous galactose tolerance tests, determination of the hepatic galactose elimination rate from elimination curves in plasma requires correction for this extrahepatic elimination.

The renal excretion of galactose in patients with normal and with reduced liver function therefore has been investigated in order to determine the magnitude of this correction. The renal excretion was studied partly under conditions of continuous intravenous galactose infusions (with constant or constantly rising plasma galactose concentrations) and partly under conditions of falling plasma concentrations (following a single intravenous injection).

Methods

The patients were studied in the morning while lying in their bed more than 12 hours after their last meal. They were allowed to drink moderate amounts of water but forced drinking to ensure high diuresis was avoided as this apparently influences the excretion of galactose.

Galactose was administered as analyzed solutions by calibrated, motor-driven syringes. Infusions of galactose were maintained for one to three hours, and the urine samples examined were collected either in periods with constant plasma concentrations, or in periods with constantly rising concentrations, depending on whether the galactose infusion rate was smaller or greater than the maximal galactose elimination rate of the patient.

In experiments with single injection of galactose about 500 mg/kg body weight was injected at a constant rate in the course of 6 minutes.

Determination of galactose in plasma was performed as previously described (TYGSTRUP *et al.* 1954). As galactose is mainly distributed in extracellular fluid (except in the liver) concentrations are expressed as mg/l plasma water.

Urine was collected through an indwelling bladder catheter. The bladder was emptied by suction, washed twice with 20 ml of sterile saline, and urine and washing fluid were mixed.

When the excretion was studied at falling or rising plasma concentrations, the galactose of the urine samples was related to the mid-concentration in plasma 8 minutes

In 2 patients a single injection of the same amount of galactose was given during high and low diuresis. After deprivation of water for 24 hours the urine flow of these patients during the test was 0.55 and 0.60 ml/min, and after administration of one liter of water in the morning 8.0 and 6.6 ml/min. The excretion of galactose was increased by 29 and 36 per cent, respectively during high diuresis.

prior to the mid-time of the urine collection interval. This correction for the average delay-time in the urinary tract was applied because in one experiment (see Fig. 2) in which the excretions were studied during rising as well as during falling plasma concentrations, application of this correction resulted in essentially identical renal clearances at comparable plasma concentrations.

For determination of galactose in urine the specimen was diluted to an estimated concentration of about 500 mg/l. The reducing power was determined by the same method as used for plasma, partly after treatment with a galactose fermenting strain of yeast, partly without treatment. The yeast, cultured in a galactose containing broth, was washed three times in saline to remove reducing substances. About 5 g of washed yeast was suspended in 30 ml of 0.2 M phosphate buffer (pH = 5.6). Two ml of diluted urine and 2 ml of yeast suspension were mixed in a flask which was stoppered, placed in a water bath at + 30 C, and automatically shaken for 90 min.

The difference between reduction values of the yeast treated and the untreated sample was taken as concentration of galactose. Treatment with yeast for determination of the unfermentable (non-galactose, non-glucose) rest reduction was found necessary especially when the concentration of galactose was low, as the rest reduction varied widely from sample to sample, and in most cases amounted to 500—1,500 mg/l of urine, expressed as galactose. The standard deviation of this analytical procedure is about 3 per cent.

As glucose is fermented by this strain of yeast as well as galactose, glucosuria will give falsely high values. In earlier experiments, when glucosuria was suspected from previous urinalysis, galactose was determined as the difference between a yeast-treated and a Notatin treated sample. In more recent experiments this procedure was used when the urine showed reaction with Notatin-impregnated paper (Clinistix®).

Results

A. *Infusion experiments with constant plasma concentration*

The rate of galactose infusion was lower than the maximal galactose elimination rate of the patients. In patients with normal liver function the infusion rate was about 300 mg/min and in patients with diseases of the liver about 200 mg/min. Consequently the plasma concentrations were lower than 400 to 500 mg/l, i.e. the level at which the eliminating mechanism becomes saturated (Tygstrup and Winkler 1954). The material comprises 32 determinations in 29 subjects with normal hepatic and renal function and 10 determinations in patients with cirrhosis of the liver or acute epidemic hepatitis.

In the subjects with normal liver function the rate of galactose excretion averaged 9 mg/min (s. d. 5.6) or 3.1 per cent of the infusion rate, and in the patients with reduced elimination of galactose 4.1 mg/min (s. d. 3.1) or 1.8 per cent of the infusion rate. Fig. 1 shows the relationship between the amount of galactose excreted in the urine per minute and the concentration in plasma water. It is seen that the correlation between the two is poor and the calculated renal clearance of galactose therefore varies widely. In patients with normal liver function it averages 35 ml/min (s. d. 18) and in the patients with hepatic diseases 17 ml/min.

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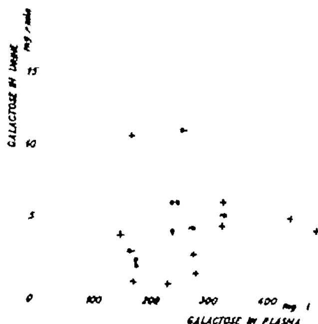


Fig. 1. Urinary excretion rate of galactose at low, constant plasma concentrations.

+ subjects with normal liver function,

● patients with disease of the liver.

•– ↓, and –• are patients in whom two successive determinations were performed.

B. Infusion experiments with rising plasma concentration

The rate of galactose infusion was between 400 and 800 mg/min which *was greater than the maximal* galactose elimination rate of the patients. The material consists of 8 subjects with normal liver function and 3 patients with cirrhosis of the liver. Most data were obtained in intervals during which the concentrations were rising at rates varying from 0.2 to 12 mg/l/min. In one case the urinary excretion during a steeper rise (34 mg/l/min) was compared to that occurring during a falling curve (— 51 mg/l/min) found after interruption of the infusion. (This experiment was used for assessment of the average delay time in the urinary tract.)

The relationship between the urinary excretion rate of galactose and the concentration in plasma water is shown in Fig. 2. In this concentration interval the correlation is more definite than at low concentrations, although the individual variation is still appreciable. Furthermore it is seen that the renal clearance tends to fall at low concentrations. In the intermediate concentration interval of 1 000 to 2,000 mg/l it averages 46 ml/min in the subjects with normal liver function, and at higher concentrations 57 ml/min.

C. Single injection experiments

The rate of galactose elimination may be calculated from the rectilinear plasma concentration-time curve by multiplying the absolute value of the slope of the curve by the volume of distribution of galactose, the latter being

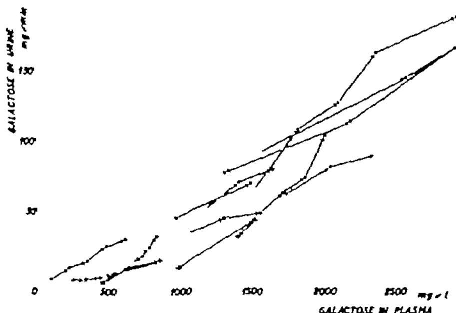


Fig. 2. Urinary excretion rate of galactose at higher changing plasma concentrations. The arrows indicate whether the observation was made during rising or falling concentration. — = subjects with normal liver function, – – = patients with cirrhosis of the liver. The dotted line indicates the amount of galactose filtered per minute at different plasma concentrations, assuming the glomerular filtration rate to be 120 ml/min.

obtained by dividing the amount injected by the extrapolated concentration at zero time. The rectilinear part of the curve, the *test period*, generally starts about 20 minutes after beginning of the injection and ends when the concentration falls below about 400 mg/l.

Quantitative collection of the urine excreted during the entire test period is not practicable, as the exact duration of this period is not known beforehand in the individual patient. In about one fourth of the experiments the test period was found to be inadequately covered by the urine collection intervals employed. Excluding these 9 experiments remained in subjects with normal liver function, and 32 in patients with cirrhosis of the liver which were satisfactory for an evaluation of the relationship between the rate of urinary excretion and the total elimination rate during the test period. (Table I.) Fig. 3 illustrates the experimental findings in two typical examples. The patients with cirrhosis were arbitrarily divided into 3 groups on the basis of the total elimination rate.

It is seen from the table that the urinary excretion rate during the test period decreases with decreasing total elimination rate, and in all 4 groups

Table 1. Urinary excretion of galactose after a single injection of about 500 mg/kg body weight in subjects with normal liver function and in patients with cirrhosis of the liver. The latter are grouped on the basis of the total elimination rate of galactose during the test period

	Amount of galactose injected	Time of plasma concentration > 100 mg/l	Collection interval of urine	Total elimination rate during test period	Average urinary excretion rate in collection interval	Excretion rate in per cent of total elimination rate	Mid-concentration of plasma in urine collection interval	Average urinary clearance in urine collection interval	Total urinary excretion	Total excretion in per cent of amount injected
	g	min	min	mg/min	mg/min	%	mg/l	ml/min	mg	
A. Normal subjects (N = 9)										
average	32.2	47	18 – 46	572	53	9.4	1,100	48	3,030	11.7
stand. dev.	6.8	8	3 7	115	17	2.6	230	14	1,130	1.7
range: min.	23.5	37	14 40	406	27	4.3	670	26	2,660	9.2
range: max.	43.0	56	22 55	732	79	14.4	1,530	74	5,530	13.2
B. Cirrhosis, total elimination rate > 300 mg/min (N = 11)										
average	29.4	67	20 – 34	356	42	11.6	1,140	35	3,090	10.5
stand. dev.	5.2	9	6 15	43	20	5.2	210	12	910	3.9
range: min.	24.6	58	16 41	304	17	5.3	840	20	1,490	4.9
range: max.	40.7	82	23 73	456	74	20.3	1,500	52	4,990	14.8
C. Cirrhosis, total elimination rate < 300, > 210 mg/min (N = 10)										
average	32.0	102	20 – 83	246	27	11.2	1,120	23	3,080	10.1
stand. dev.	6.1	15	3 12	29	13	6.3	150	10	1,370	6.2
range: min.	26.5	69	16 72	21[illegible]	10	3.8	890	9	1,290	3.7
range: max.	46.8	128	24 113	298	52	24.1	1,300	44	6,660	23.1
D. Cirrhosis, total elimination rate < 210 mg/min (N = 11)										
average	29.5	120	20 – 89	188	23	11.9	1,100	21	2,310	8.3
stand. dev.	3.8	26	2 30	19	11	5.2	1[illegible]0	11	930	3.6
range: min.	23.0	91	17 71	156	3	1.7	835	2	950	2.3
range: max.	37.6	179	24 178	209	43	20.7	1,380	41	3,910	15.2

averages about 10 per cent of the latter. As the mid-concentrations of the groups in the sampling interval are almost identical, the values of renal clearance of galactose also show a decrease roughly parallel to the fall in total elimination rate.

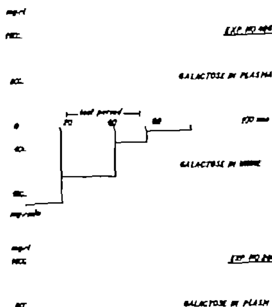


Fig. 3. Plasma galactose curv and urinary excretion in subject with normal liver function and in patien with cirrhosis (belonging to group C of Table I) fter single intravenous injection. The amount of galactose ex creted in the second and third urine sample was used for calculation of the average excretion rate during the test period.

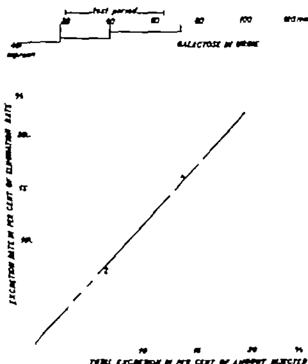


Fig. 4. Correlation between the average urinary excretion rate during the collection interval in per cent of the total elimination rate during the test period and the total urinary ex cretion in per cent of the amount injected.

– subjects with normal liver function,

– patients ith cirrhosis and total elimination rate greater than 300 mg/min,

– patients with cirrhosis and total elimination rate between 300 and 210 mg/min,

– patients ith cirrhosis and total elimination rate smaller than 210 mg/min. The line shows the average relationship between the values in all the patients.

The total amount of galactose recovered in the urine is of the same order of magnitude in all groups. The ratio between the amount excreted and the amount injected is found to be correlated to the ratio between the urinary excretion rate and the total elimination rate during the test period. This correlation is depicted in figure 4 the correlation coefficient (r) is + 0.80. More specifically the correlation may be expressed as

$$\frac{\text{total excretion}}{\text{amount injected}} \times 1.1 = \frac{\text{urinary excretion rate}}{\text{total elimination rate.}}$$

This means that the average urinary excretion rate during the test period may be computed from the total excretion, *i. e.* without catheterisation of the bladder The standard deviation of the factor 1.1 is 0.3

Discussion

The data presented agree with the concept of reabsorption of galactose in the renal tubules (GAMMELTOFT and KJERULF JENSEN 1943) with a low and incomplete threshold at a concentration in the body of 100 to 200 mg/l. The relatively slow rise in clearance at higher concentrations indicates that the T_m of the process is very high, unless the reabsorption at these high concentrations is mainly passive, *i. e.* by diffusion. DOMINGUEZ and POMERENE (1944) found that the galactosuria was highly dependent on the rate of urine flow our data indicate that this is the case to some extent, while GAMMELTOFT and KJERULF JENSEN (1943) were unable to demonstrate any such relationship The reason for this discrepancy may be sought in species differences, in different procedures used for production of diuresis, and in different plasma concentration levels studied Recently WALDSTEIN *et al.* (1960) found that renal galactose clearances of the same order of magnitude as the glomerular filtration rate may be obtained during water diuresis.

In the usual peroral galactose tolerance tests in which the amount of galactose excreted in the urine is the parameter observed, an abnormal renal handling of galactose may be an important source of false interpretations. In intravenous tests based on the disappearance of galactose from the blood, changes in urinary excretion will only moderately influence the result, the degree of influence somewhat depending on the type of test used.

If galactose is infused at rates lower than the maximal galactose elimination rate of the liver as for determination of galactose blood clearance (TYGSTRUP and WINKLER 1958) the urinary loss may be disregarded. When larger amounts are infused the plasma concentration, and thereby the urinary excretion, rises, and as the hepatic elimination is constant, the relative urinary loss rises as well At a concentration in the body of for instance 2 000 mg/l it will amount to about 20 per cent of a normal maximal hepatic galactose elimination rate of 500 mg/min. Under these conditions the latter only can be determined if the urinary loss is corrected for

Technically single injection of galactose is the simplest form of intravenous galactose tolerance test and should be well suited for practical assessment of the liver function in terms of maximal hepatic galactose elimination rate.

Calculation of this value from the rectilinear slope of the plasma galactose concentration-time curve and the average urinary excretion rate during the test period requires certain assumptions. Thus it must be assumed that other extrahepatic routes of galactose elimination than renal excretion are negligible, and that the apparent volume of distribution of galactose remains constant during the test period.

Since the rate of urinary loss of galactose decreases with decreasing plasma concentrations, one should expect some deviation from rectilinearity of the plasma galactose concentration-time curve (see appendix). Generally this deviation is not discernible, however, probably because it is hidden by the analytical error, and even when a number of curves are pooled and analyzed (TYGSTRUP and WINKLER 1954) there is only a slight and statistically insignificant deviation. The assumption of a constant excretion rate during the test period, used for the correction of the total elimination rate, only is justified if the excretion rate is small in proportion to the total elimination rate. From this point of view it is fortunate that the renal clearance of galactose generally is decreased in patients with cirrhosis of the liver.

Whether the diminished excretion in patients with cirrhosis is due to diminished filtration rate of galactose or to increased rate of reabsorption cannot be stated, as determination of these parameters have not been included in this study. Reduction of the glomerular filtration rate to about 50 per cent of normal has been demonstrated in patients with decompensated cirrhosis of the liver (ÖHEM 1960), and if tubular reabsorption of galactose is unchanged, this may partly account for the diminished excretion. Furthermore the rate of urine flow in the cirrhotics was on an average slightly smaller than in the normals, a fact which may have enhanced back-diffusion of galactose in the tubules.

The reduced renal clearance of galactose may explain why negative results of peroral galactose tolerance tests, based on the quantity of galactose excreted in the urine, are frequently encountered in patients with cirrhosis of the liver.

Appendix

(In collaboration with Chr. Oram, M. Sc.)

The galactose plasma concentration-time curve following single injection may be divided into 3 parts (TYGSTRUP and WINKLER 1954) (Fig. 5.)

If the disappearance of galactose from the plasma during the test period is exclusively determined by hepatic elimination and renal excretion, and if hepatic elimination rate and renal clearance are presumed to be constant in this interval, the curve is described by the equation

$$\frac{dx}{dt} = -\frac{GE}{V} - \frac{Cl\,x}{V} \quad (1)$$

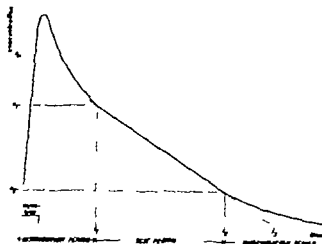


Fig. 5. Theoretical plasma galactose concentration-time curve following single intravenous injection of 500 mg/kg body weight (TYGSTRUP and WINKLER 1954). The broken lines show the extrapolations and projections used for the calculations.

where is concentration, t is time, GE is maximal hepatic galactose elimination rate, Cl is urinary clearance, and V is volume of distribution. Therefore

$$= \left(\;\; + \frac{GE}{Cl} \right) \qquad - \frac{Cl}{V} \quad (t - t') \qquad - \frac{GE}{Cl} \tag{2}$$

where is the base of natural logarithms, and c' and t' are corresponding values of concentration and time, e.g. and t in Fig. 5.

The shape of the curve described by equation (2) depends on the relative magnitude of the constants. In order to evaluate the deviation of the curve from rectilinearity in normals and cirrhotics, the curves of Fig. 6 were constructed. The constants used for the special solutions depicted in the figure were obtained by calculating average values of GE, Cl, and V from the material of group A and group C in Table 1 (curve I and curve II respectively). Curve I therefore may be taken to represent an average normal subject, and curve II an average patient with cirrhosis of the liver. A number of concentrations were calculated for values of t greater than 20 min and greater than 400 mg/l covering the usual test period. These concentrations, with the standard deviation of the analysis, are plotted in Fig. 6. From each series of points the best fitting straight line was computed by regression.

Fig. 6 shows that in both cases examined the deviation of the straight line from the calculated concentrations is smaller than the standard deviation of the analytical procedure. For practical purposes it therefore is warranted to regard the total elimination rate in this period as constant.

Making use of this approximation equation (1) may be written

$$g = -\frac{GE}{V} - \frac{L}{(t_2 - t_1) \quad V} \tag{3}$$

where g is the slope of the best fitting straight line during the test period, and L is the amount excreted in the urine in this period.

The volume of distribution (V) may be calculated from the concentration after distribution has taken place (c_0) and the amount present in the body at that time (A) (cf. Fig. 5)

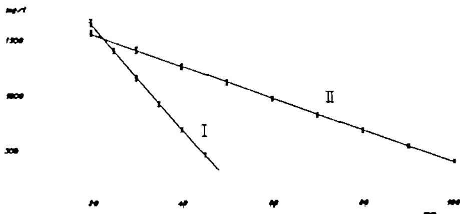


Fig. 6. The influence of the kinetics of urinary excretion of galactose on the plasma concentration-time curve in an average normal subject (curve I) and in an average patient with cirrhosis (curve II). The concentrations shown (+) were calculated from equation (2) assuming the urinary excretion rate to be proportional to the plasma concentration, and the straight line was calculated by regression from the same concentrations, assuming the urinary excretion rate to be constant according to equation (3). The deviation of the straight line from the calculated concentrations is seen to be smaller than the standard deviation of the analytical procedure, depicted by horizontal bars. (S. D. = 0.01 × + 10 mg/L)

$$V = \frac{M - GE \times t - U}{\;} \tag{4}$$

where M is the amount injected, and U is the amount excreted in the urine in the distribution period. Eliminating GE from equation (3) and (4) we get

$$V = \frac{M - \left(U - \frac{L}{t_2 - t} \quad t\right)}{c_1 - g \quad t} \tag{5}$$

where the denominator $- g \times t$ equals c_t (cf. Fig. 5).

From the material of Table I it was calculated that on an average

$$U - \frac{L}{t_2 - t} \quad t = 670 \text{ mg or } 0.02 \quad M$$

and applying this value equation (5) may be written

$$V = \frac{0.98 \quad M}{\;} \tag{6}$$

From equations (3) and (6) it appears that

$$GE = -g \quad \frac{0.98 \quad M}{\;} - \frac{L}{t_2 - t_1} \tag{7}$$

It was demonstrated (Fig. 4) that on an average

$$\frac{U}{t_0 - t} \times \frac{c_0}{-g \times M} = 1.1 \times \frac{U_{total}}{M}$$

where U_{total} is the amount excreted in the urine during the whole experiment, and applying this expression in equation (7) we get

$$GE = \frac{-g}{c_0} \times (0.98 \times M - 1.1 \times U_{total}) \qquad (8)$$

The factor $\frac{-g}{c_0}$ equals $\frac{1}{t}$ and t_0 may be determined graphically from the curve. (*cf.* Fig. 5)

If the factors 0.98 and 1.1 in equation (8) are omitted, *GE* on an average will be overestimated by 3 per cent. As this error is unimportant in clinical work, and may be overshadowed by the individual variations of the factors, the equation

$$GE = \frac{M - U_{total}}{t} \qquad (9)$$

may be preferred for this purpose.

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Acta physiol. scand. 1961 51 275—282

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The Effect of Juvenile Hormone on the Respiratory Metabolism of Silkworm Pupae, as Recorded with a New Semi Micro Device

By

JOHAN B. STEEN

Received 21 November 1960

Abstract

STEEN J. B. *The effect of juvenile hormone on the respiratory metabolism of silkworm pupae, as recorded with new semi-micro device.* Acta physiol.scand. 1961 *51* 275—282. — The respiration of Polyphemus pupae during normal pupal-adult development has been compared to that during abnormal development as induced by juvenile hormone. Respiration was measured as oxygen-uptake with a new semi-micro recording spirometer. Striking differences were found and it is suggested that the respiratory response may be used as a sensitive assay for the juvenile hormone.

During the time required for an insect to develop from a pupa to an adult, the metabolism, as signalled by the rate of oxygen consumption, increases systematically. This has been documented for several species and orders of insects, particularly in the case of the Cecropia silkworm (SCHNEIDERMAN and WILLIAMS 1953). If such a pupa is injected with juvenile hormone (WILLIAMS 1959), it will not undergo the normal metamorphosis but instead develop into an individual which exhibits a mosaic of juvenil and imaginal characters or even into a second pupa. The object of the present investigation was to compare the respiration of animals following these two paths of development and thereby gain insight into some of the metabolic effects of the juvenile hormone. To this end semi-micro self compensating respirometer was developed, capable of recording the oxygen consumption continuously over several weeks with an accuracy of some 5 per 5 ml of gas consumed.

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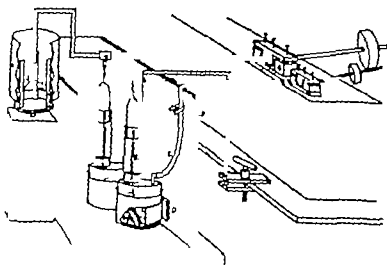


Fig. 1 Device for continuous measurement of oxygen uptake. The total length of the arm from pen to fulcrum is 60 cm, the rest is in proportion to this except for the respiratory chamber which is somewhat enlarged

A = Respiratory chamber	E = Spirometer	I = Drum
B = Citric acid jacket	F = Weights	J = Tripod
C = Side arm	G = Fulcrum	K = Revolving disc
D = Chamber rack	H = Lever arm	L = Time drive

Materials and Methods

1 *Experimental animals*

The present investigation is based on measurements on a total of 8 female pupae of the giant moth *Antheraea polyphemus*, (a close relative of the Cecropia silkworm) varying in weight from 4.0 to 5.5 g. They had all been stored at 6° C, for about one half year so that they would develop promptly when transferred to room temperature

2. *Recording spirometer*

This device was designed as a simple and inexpensive recorder for oxygen-uptake in larger insects. The accuracy over short periods of time (two to four hours) is poor but over longer periods the accuracy is fully sufficient to record constant or systematically changing rates of oxygen-uptake. The device described by Brown (1957) for the same purpose requires very accurate temperature and pressure regulation, whereas the device to be described here compensates automatically for external variations in temperature and pressure within 1° C and 15 mm Hg

a. *Principle*

Two floating respirometers, communicating separately with identical respiratory chambers, govern the position of a pen and a drum respectively. The animal is placed in the chamber connected to the pen. Both chambers contain carbon dioxide absorbent. The curve drawn by the pen on the drum is, ideally, a record of only the volume change due to the animal since variations in temperature and pressure will influence the position of both pen and drum equally and will not lead to net movement of the pen on the drum.

b. *Apparatus*

The apparatus consists of respiratory chambers fastened in a thermoregulated water bath and lever arms each with a fulcrum, a counterweight, a respirometer and a pen or a drum (See Fig. 1) For maximum sensitivity it is essential that the arms and anything ttached to them, except the counterweights, be as light as possible.

It is convenient to ecord from at least two respirometers on the same drum. 300 ml glass jars worked satisfactorily as respiratory chambers for single silkworm pupae. A wet carbon dioxide absorbent \ KOH is placed on the bottom and separated from the animal by non-metallic screen. The chambers are closed off with two-hol rubber stopper A section of bent glass tubing is inserted as side-arm into one hole and into the other hole, bollow thick-wall section of Lucite tubing The latter fits tightly into a second rubber stopper which makes a flexible joint with section of this glass tubing going up into the espirometer A section of large diameter glass tubing fits around this stopper forming jacket. The jacket is filled with a saturated sol tion of citric acid which has favourable surface properties and very low solubility for oxygen[1]

Thin-walled plastic centrifuge tubes (10 to 40 ml) serve as respirometers nd are suspended from the light aluminum lever rms by wire clips so that they float in the citric acid. The pen fastened at the end of the arm touches the drum as lightly as possible and the counterweight is adjusted so that the lever arms are in equilibrium at any position of the spirometers. The change in buoyancy of the spirometers is compensated by an upward bend of bout 8 — 12 degrees in the rod that carries the weight. In order to make the arm more sensitive the compensator arm is bent as shown in Fig 1 so that the center of gravity of the drum lies on the line through the counterweight and the fulcrum.

The fulcrums are mad of wood or aluminum and rest on hard surface by means of tw pointed screws. These tips are on level with the center of the arm. The fulcrums are placed so that the arms are horizontal when the spirometers are half submerged.

The light recording drum is suspended from the arm by silk thread and a spring wire tripod (Fig. 1 — J) the ends of which are bent slightly outw rd to fit into holes drilled in the lower end of the drum. The pex of the tripod is in the center of the drum and the feet rest against three ertical glass rods fastened to clock motor (Gorrell type MO — 1000) Contact at this point is chieved by winding the silk thread a few times before the drum is fitted over the rods The friction imposed by the glass rods upon the ertical motion of the drum is overcome by the ibration from the motor

T attain simila magnification the distance between fulcrum and spirometer is identical on all arms as is the distance from the spirometer to the pen (or the drum)

The recording paper has ertical lines and is changed most easily by first disconnecting the spirometer from the arm so that the latter swings up. After each refilling or change of paper the level of citric acid should still be the same

c. *Calibration*

The spirometers are calibrated by injecting gas from syringe through the side-arm hile the pen records on drum which is turned by hand. \ scale is mad so that it reads the olumes t 0 C directly Each ml on this scale occupies $\frac{X \quad T}{273}$ cm where X is the displacemen of the pen caused by the injection of one ml t temperature T The same calibration is ccomplished w th the lever arms in horizontal position. I order to project curves properly onto this base line is convenien make ruler with a straight back edge and front edge which conforms to the arc traced out by the pen.

[1] Polarographic anal ses, sensiti to 10^{-4} M failed to detect any oxygen in saturated citric acid well shaken with oxygen (H. Ritter, personal communication)

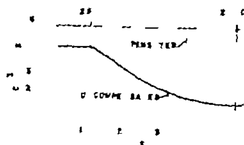


Fig. 2. The two curves illustrate the efficiency of the temperature compensation. They are recorded simultaneously during a temperature fall of 4° C. in the respiratory chamber but on separate drums. The upper curve is recorded with the device shown in Fig. 1, the lower curve is recorded on a horizontally stationary revolving drum. The dotted line at the top is the base line.

d. *Accuracy of the method*

Gas samples which were withdrawn from the respiratory chambers during experiments and analyzed on the SCHOLANDER 1/2 ml gas analyzer (SCHOLANDER 1947) gave less than 0.03 % CO_2.

The sensitivity can be varied using spirometers with varying diameter. Extremely small spirometers must be avoided, however, since they tend to cling to the tube from the animal chamber. With a spirometer diameter of 1 cm an injection of no more than 0.05 ml of gas can be detected on the drum. This amount of gas is equal to about 0.5 mm vertical movement of the pen.

Variations in the atmospheric pressure of up to 40 to 50 mm Hg are not recorded on the compensated drum; with larger variation the compensation becomes less complete.

Fig. 2 shows recordings during a temperature drop in the waterbath of 4° C. One curve was recorded on the compensated drum, the other on a regular drum. The deflections are 0.1 ml and 3.6 ml respectively. With the waterbath regulated to within 1° C the error introduced by this imperfectness is 0.025 ml.

Repeated gas injections gave an accuracy in the recordings of 10 % for 1 ml samples, 4 % for 5 ml samples and 2 % for 10 ml samples. This uncertainty is due to imperfectness in the balance of the lever arm, to variation in vapor and surface tension and to difficulties in reading the recordings accurately. The accuracy during actual runs is probably no more than 5 % per 5 ml of oxygen consumed. A giant insect pupa uses about 5 ml over 48 hours, which means that values calculated from this on an hourly basis using this method are accurate to about 5 μl. The Warburg manometers are, for comparison, ten times more accurate.

Values for oxygen consumption should be corrected to 760 mm Hg, using the average pressure during the recording time. No humidity correction is needed.

Results

The pupae were anesthetized with CO_2 for 15 to 30 min immediately after they had been removed from the cold room. Six of them received 100 μl of a suspension of juvenile hormone in peanut oil by injection through the mesothoracic tergum just lateral to the midline. Two pupae served as controls, one received 100 μl peanut oil, the other was not injected at all. The small number of controls is sufficient since the normal developmental respiration resembles closely that of the related Cecropia pupa which has been well investigated (SCHNEIDERMAN and WILLIAMS 1953). Seven of the pupae initiated development immediately after they had been removed from the cold room, the peanut oil

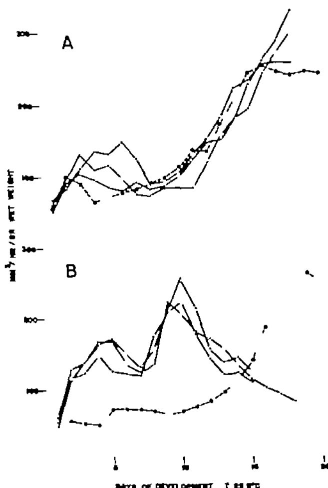


Fig. 3. Rates of oxygen-uptake during development in the Polyphemus silkworms. (*A*) The respiration of three pupae which were injected with Juvenile hormone (solid lines) but which developed normally is compared with that of pupa which was not injected at all (broken line). (*B*) The respiration of three pupae that received injections of juvenile hormone and developed into individuals which had retained several pupal characteristics (solid lines) is compared with that of the pupa which received an injection of pure peanut oil (broken line).

injected pupa started two to three days later. This is well within the range commonly experienced in this species.

Respiratory measurements were started within an hour after injection. The temperature was kept at 25.5 ± 0.5 C, throughout the experiment. When the chambers had been properly assembled and fastened to the clips in the waterbath, they were aerated through the side arm with the disconnected respirometers resting on the citric acid. The side arms were left open to let the

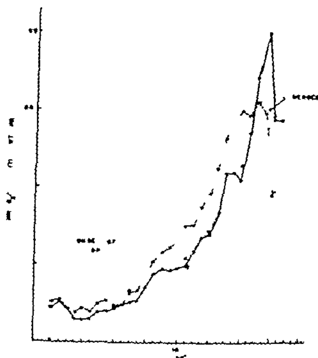


Fig 4. Rates of oxygen-uptake of three Cecropia pupae during normal pupal-adult development. The dotted curve is taken from Schneiderman and Williams (1953) the two others were obtained with the technique presented above

spirometers sink and push air out. The side arms were then closed and oxygen bubbled through the citric acid and into the respirometers via a J-shaped glass tubing The recording was interrupted once or twice to renew the carbon dioxide absorbent and to check the stage of development of the pupae When the animals had finished development, they were scored as to juvenile and imaginal characters according to the morphological bioassay of WILLIAMS (1959)

I The oxygen uptake of Polyphemus during normal adult development

In Fig 3 A the dotted curve records the characteristic time course in the oxygen consumption of the uninjected *Polyphemus* pupa during the course of development at 25 5 C. The shape of the curve is much like that for Cecropia (Fig 4) except the time is slightly shorter (16 to 17 days as compared to 21 to 22 days) The slight increase in oxygenconsumption during the first few days resembles that in Cecropia and is probably a reaction to the temperature shock the animal experiences when removed from the cold

Except for the absence of the initial hump the peanut oil injected pupa showed neither morphological nor metabolic differences compared to the uninjected pupa (Fig. 3 B, dotted curve)

2. *The oxygen uptake of Polyphemus developing in the presence of juvenile hormone*

Despite the fact that the juvenile hormone had been found very potent in earlier tests, only three out of the six injected pupae showed a morphologically abnormal development. The three affected pupae had retained several pupal characteristics at the end of metamorphosis. For example, they displayed pupal cuticle and antennae and they had failed to develop wing muscles. The time course of oxygen-uptake during this development was very different from the normal picture as demonstrated in Fig. 3-B, although the total uptake of oxygen was not much different. The initial hump is much larger and lasts twice as long as in the normal developing adult and it goes directly over into a second hump which reaches a maximum around the 11th day of development. At this time the morphological development is terminated and the curves fall off gradually returning to the initial level on about the 20th day.

Fig. 3-A (full drawn curves) records the rate of oxygen consumption for the pupae that received active hormone but nevertheless developed into morphologically normal adults. During the first week of development the respiration resembles that of the affected pupae although the initial hump is somewhat lower; during the later part it duplicates that for normal development. This indicates that while the hormone was not active enough to induce morphological irregularities, it was strong enough to induce metabolic deviations.

Discussion

The possibility of using the occurrence of a pronounced initial hump as a bioassay is suggested. At present a safe judgment cannot be passed as to how dependable this test would be.

A diapausing pupa will normally respond to any injury, even to a needle prick through the cuticle, with a temporarily elevated respiration (SCHNEIDERMAN and WILLIAMS 1953). Such an injury metabolism, however, cannot be induced in normally developing animals (HARVEY, personal communication) and it was not found in the pupa injected with pure peanut oil. This does not remove the possibility that the presence of juvenile hormone may increase the sensitivity towards injury or toward a temperature shock under these circumstances.

During the development of the respirometer I benefited from the expert advice and craftsmanship as well as patience of Mr. R. CHAPMAN. I wish to thank Dr. C. M. WILLIAMS, who kindly supplied both experimental animals and the hormone and who executed the bioassays. I am much indebted to Dr. W. R. HARVEY for his interest and support during all stages of this investigation. The investigation was supported by the Norwegian Research Council and through grants to Drs C. M. WILLIAMS and W. R. HARVEY from the United States Public Health Serv

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Conduction Rates of Afferent Fibres to the Anterior Tongue of the Dog

By

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Abstract

Iriuchijima, J. and Y. Zotterman. *Conduction rates of afferent fibres to the anterior tongue of the dog.* Acta physiol. scand. 1961. *51.* 283—289. — Rate of conduction and sensory function were studied in single afferent fibres of the chorda tympani and the lingual nerve of the dog. All taste fibres appeared to be medullated fibres (A δ) conducting at rates from 18 to 2 m/sec. The thermal fibres, "cold" as well as the more rare "warm" fibres responding to slight temperature changes (less than 1° C) were also A δ fibres. All "touch" fibres so far encountered were found to be medullated A fibres. In addition to these medullated fibres some afferent C fibres were found which responded to heavy pressure as well as to extreme temperatures. These fibres obviously serve nociceptive reactions.

Single fibre analysis of mammalian cutaneous nerves has been made by Maruhashi, Mizuguchi and Tasaki (1952) for the myelinated nerve fibres and by Iggo (1959, 1960), Iriuchijima and Zotterman (1960) and Hensel, Iggo and Witt (1960) for the unmyelinated. In these recent papers the specificity of afferent cutaneous C fibres has been established which suggests that, in the cutaneous sensation, a very elaborate, discriminative rôle is played by C fibres.

From the special rôle played by the tongue in taste the fibre spectrum of the chorda tympani and the lingual nerve was suspected to be different from that of the cutaneous nerve. In the present study rate of conduction and sensory function were studied in single afferent fibres of these nerves.

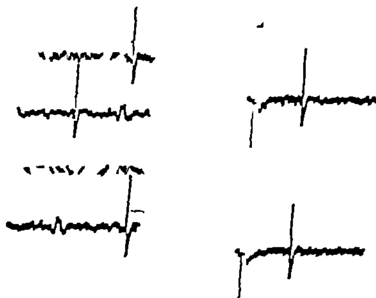


Fig. 1. Determination of conduction velocity. Left: Action potentials of a salt fibre evoked by pouring 0.5 M NaCl solution on the tongue. Right: Evoked electrical stimulus applied to the receptive field. Conduction distance 125 mm. Time in msec. Conduction velocity was determined as 10.4 m/sec.

Materials and Methods

A total of 16 mongrel dogs of both sexes, weighing between 2.2—11.5 kg, were used in this study. After basal anesthesia with 10 mg Tetropoonm and 0.4 mg Scopolamine hydrobromid subcutaneously injected, the animals were anesthetized by injection of 6 per cent Nebumalnatrium solution containing 1.8 g Pentobarbitone, 4 g Pentobarbitone sodium and 25 g urethane in 10 ml. The initial dose was 0.4 ml of this solution per kg body weight. Additional injections were made to maintain the suitable depth of anesthesia by means of [illegible]enous cannula.

After removal of the posterior part of the mandible the chorda tympani was exposed, cut centrally and placed on a black plate in a paraffin pool. The nerve was desheathed and split into such thin strands that even electrical response of C fibres could be recorded. The experiment on the lingual nerve was also made in the same way on the peripheral part of the nerve, below the point where the chorda tympani joins the lingual nerve. The compound action potential was recorded by placing leads on the chorda tympani in its extra tympanic course as well as on its tympanic part in the middle ear. The stimulating electrodes were placed on the peripheral part of the lingual nerve close to its entrance into the tongue at [illegible] distance from the submaxillary chorda tympani. Records were also made when stimulating this latter nerve. For the sake of comparison, leads were also placed on the central part of the lingual nerve above the entrance of the chorda in order to measure the speed of the fastest A fibres of the trigeminal system.

For taste stimulation, water, 0.5 M NaCl, 0.5 M sucrose, 0.01 M quinine hydrochlorid solution, and 0.5 M acetic acid were poured on the tongue. The last mentioned three substances were dissolved in Ringer solution to avoid the interference of the water response. Whenever a strand was found to contain a fibre responding to any of the above stated test solutions, the receptive field was determined with a small cotton ball soaked with the solution to which the fibre was responding. In search for the thermoreceptors

Table I Conduction velocities of the afferent fibres in the chorda tympani and the lingual nerve of the dog (in m/sec)

Salt fibres	Sweet fibres	Bitter fibres	Acid fibres	Water fibres
1.9	2.0	1.6	6.2	3.5
6.2	4.5	2.8	9.0	3.7
8.7	4.8	3.6	10.0	6.8
10.0	5.5	4.7	12.8	7.3
10.4	12.2		15.3	8.3
13.0	13.7		16.3	9.5
13.0	15.2			10.9
14.4				17.8
14.5				

Warm fibres	Cold fibres	Touch fibres		Pressure fibres	
1.9	Ling. n.	2.9	Ling	0.7	Ling. n.
12.0	5.2	6.5	4.1	0.8	0.4
13.3	7.7	7.1	4.7	0.9	0.5
	8.9	7.5	7.0	1.7	0.7
	10.0	8.3	13.2	1.8	0.7
	10.8	12.5	15.5	2.0	0.8
	11.0	15.0	*23.0	4.8	0.8
	11.3	16.4		5.4	0.9
	11.4	24.0		6.2	5.6
				6.5	7.0
				6.8	16.2
				6.9	
				7.4	
				8.2	

Some responded also to salt   Responded also to cooling

fibres a conventional thermode was used and the receptive field was localized by a warmed or cooled metal rod. For mechanical stimulation the tongue was touched with the tip of a brush or pressed with the rounded tip of a glass rod. If the receptive field was determined accurately it was always possible to stimulate it electrically for the measurement of conduction velocity at such a weak intensity as did not result in the movement of the tongue. The identity of the electrically evoked spike was ascertained by the identical height and configuration of the spikes produced by the natural stimulation using the same speed of recording, as will be seen from Fig. 1

Results

Taste fibres Dissection of taste fibres was performed exclusively in the chorda tympani. As is shown in Table I where all the fibres on which the measurement of conduction velocity was made are found, most taste fibres fell in the category

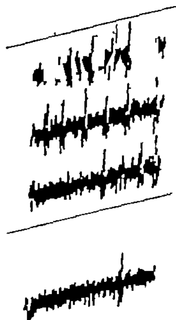


Fig. 2 Afferent C fibre in the lingual nerve. Upper Response to strong pressure with glass rod Lower Electrically evoked action potential. Conduction distance 80 mm. Time in 100 msec. Conduction velocity 0.8 m/sec.

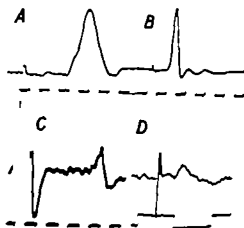


Fig. 3 Compound action potentials
A. Record from the chorda in the middle ear stimulating electrodes on the lingual nerve as it enters into the tongue conduction distance 62 mm. Time in msec fastest fibres 30 m/sec.
B. Same preparation; leads on the lingual nerve stem centrally of the chorda tympani conduction distance 57 mm. Time in msec. Conduction rate of fastest fibres 60 m/sec.
C. Same preparation chorda tympani, C elevation. Time in 10 msec. Conduction distance 62 mm. Speed of conduction 1.5—0.8 m/sec
D Recording leads on the chorda tympani stimulating electrodes on the sub-maxillary chorda tympani. Conduction distance 18 mm Time in 10 m/sec. Rate of conduction 11—4 m/sec

of the A δ group from conduction velocity Some of them presented conduction velocities lower than 2 m sec, which is generally assumed to be the upper limit of C fibres. Nevertheless we hesitate to regard them as C fibres, because as the tongue was stretched and exposed to room temperature, slower rates might be obtained than under normal conditions, though care was taken to keep the rectal temperature of the animal as well as the paraffin pool at around 38.5 C.

Among the taste fibres, the fibres responding to quinine solution presented especially slow conduction rates as compared with the other kinds of taste fibres. This observation is in good agreement with a previous observation (ANDERSSON et al. 1949) that the response to bitter substances is generally built up by smaller spikes, which was also confirmed in this study Taste fibres did not respond to the pressures which excited the "pressure" fibres.

Thermal fibres. Determination of conduction rate was possible only for three warm fibres in the chorda tympani Some more warm fibres responding to heating the tongue were observed but were lost when we tried further splitting

for better identification. Of the three fibres, which responded to slight temperature changes (less than 1 C) two conducted at rather rapid rates and presented action potentials of relatively large amplitudes. On the other hand, the conduction rate of the third one was very slow (1.9 m/sec) and the action potential was also relatively small in amplitude. However from the same reasons as stated before this fibre probably did not belong to C fibre group.

Cold fibres were found abundantly in the lingual nerve. Their rate varied from 3.2—11.4 m/sec. Those which are listed under the item of "cold" fibre in Table I showed highly sensitive responses to cooling (less than 1 C) and did not respond to touch or pressure. Some fibres which responded to cooling were found to be sensitive also to touch and are listed under the term of touch fibre with a special note.

Touch and pressure fibres In this study the fibres responding to light touch with the tip of a brush are referred to as "touch" fibres and those which could be stimulated only by strong pressure with a glass rod and not by the brush were referred to as "pressure" fibres. All touch" fibres conducted at the rate of the A class fibres no matter whether they were found in the chorda tympani or in the lingual nerve. As stated above, some "touch" fibres also responded to moderate cooling

In addition to these A class fibres we found 10 high threshold mechanoceptive fibres, 3 in the chorda tympani and 7 in the lingual nerve, conducting at rates slower than 1 m/sec. The response to strong pressure with a glass rod of one of this kind of fibres is presented in Fig 2 as well as the action potential evoked by an electrical shock applied to the receptive field for determining the conduction velocity These fibres also responded to extreme warmth and cold, over 45 C and below 20 C starting from 37° C, just as the cutaneous pressure C fibres did (cf. Fig 10 of the authors preceding paper) Some of them, found and localized by applying pressure on the tongue, did not respond to extreme temperature changes on the upper surface of the tongue. However when the thermode was placed on the lower surface of the tongue, the response to extreme warming and cooling was easily obtained, indicating that these fibres were supplying the lower surface of the tongue.

Typical records of the compound action potential of the chorda tympani will be seen in Fig 3 The fastest fibres were found to conduct at rates of about 30 m/sec while the fastest trigeminal fibres of the lingual nerve were found to conduct at rates of about 80 m/sec. A C-wave was invariably recorded conducting at rates of 0.8 to 1.5 m/sec when the distal part of the lingual nerve was stimulated. Stimulation of the submaxillary chorda tympani yielded one wave only conducting at rates of 4 to 11 m/sec (Fig 3 D)

Discussion

According to FOGLEY (1945) 18 per cent of the afferent fibres of the chorda tympani of the cat are unmyelinated while in the dog they constitute 23

cent. In comparison with cutaneous nerves the chorda tympani thus contains relatively very few C fibres. Our finding of only 3 single fibres in the chorda conducting at rates below 1 m/sec out of a total of 66 examined chorda fibres seems thus to be in accordance with what could be expected. Besides these 10 fibres which undoubtedly were afferent C fibres we found altogether 6 afferent fibres conducting at rates below 2 m/sec and additionally 5 fibres conducting at rates of between 2 and 4 m/sec. It is very likely that these fibres are of small diameters around or below 1.5 μ with rather underdeveloped sheaths and therefore might have been counted as unmyelinated by the histologists. The finding that the fastest afferent fibres of the chorda tympani conducted at rates of about 30 m/sec is in accordance with the histological finding that the diameters of the medullated fibres of the chorda tympani in the dog do not exceed 6 μ in diameter (ANDERSSON et al. 1950) but is considerably lower than the rates around 50 m/sec found by COHEN et al. (1957) for the fastest fibres of the chorda tympani of the cat. The conduction rates of the B wave, 4 to 11 m/sec, elicited by stimulating the preganglionic secretory fibres running to the submaxillary gland, however agreed very well with the values of 4 to 12 m/sec found by these authors in the cat.

Stimulation of the submaxillary branch of the chorda tympani nerve gave no C wave which was invariably recorded from the central part of the chorda tympani when the lingual nerve was stimulated. This finding is in accordance also with Foley's finding (l. c.) that removal of the superior cervical ganglion does not result in any diminution in the unmyelinated axons in the chorda tympani of the cat. Thus the C wave recorded by us from the central part of the chorda tympani must be due to the activity of afferent C fibres only

The largest fibres of the chorda tympani are obviously mechanoceptive. The great number of fibres conducting at rates from 2 to 10 m/sec found in the chorda may to a certain extent depend upon that we used young puppies. But the compound action potential of the chorda gave much the same rates of conduction for the A elevations, as well as for the C wave, in the adult dog Thus some of the quite small medullated fibres may loose their myelin sheath earlier in their peripheral course and should thus render rather low rates when stimulated from the surface of the tongue.

GASSER (1960) has recently drawn attention to the fact that the speed of the delta elevation comes out to be slower than the rate computed from their diameters, based on the assumption of a linear relationship between velocity of conduction and fibre diameter It is obvious that the chorda tympani containing relatively so many more medullated fibres of very low conduction speed than cutaneous nerves in this respect seems to be related to the visceral nerves such as the vagus, which in the cat contains thoracic afferent fibres conducting at rates of 2 to 10 m/sec (PAINTAL 1953) Both in the cutaneous and the visceral nerves this elevation has been attributed to A δ fibres.

Only three "warm" fibres were encountered in the chorda tympani. Two

of them were obviously medullated fibres conducting at about the same average rate as that of the "cold" fibres of the lingual nerve. One "warm" fibre conducted, however, at a rate as low as 1.9 m/sec. Contrary to our recent finding of numerous "specific cold" C fibres in cutaneous nerves (Iriuchijima and Zotterman 1960) we have not found so far any "cold" fibres conducting at C rates in the lingual nerve. The afferent C fibres which we have been able to trace hitherto seem all to be high-threshold mechanoceptive fibres which to a great extent also respond when their endings are exposed to extreme temperatures.

The taste fibres display a great variety of conduction rates. The highest rate 17.8 m/sec determined for a "water" fibre while for the "bitter" fibres (only 4 fibres tested) the velocities were found to be below 5 m/sec. We do not claim that our failure so far to find any taste or thermal fibres conducting at rates below 1 m/sec proves that in the tongue these modalities are not served by any C fibres. However, as we have applied the same technique with which we were able to record the activities of specific C fibres in cutaneous nerves, and since in this research we actually recorded the activity of afferent C fibres in the lingual nerve as well as in the chorda tympani, we are inclined to assume that taste as well as thermal sensations from the tongue are mediated principally if not totally by medullated fibres of small diameters.

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X Ray Diffraction Studies on Peripheral Nerve Myelin

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Abstract

HÖGLUND, G and H. RINGERTZ. *X-ray diffraction studies on peripheral nerve myelin.* Acta physiol. scand. 1961 *51* 290—295. — *The dimension* of the radial repeating unit was measured on peripheral nerve from 15 species belonging to 4 vertebrate classes. I the fishes the dimension was 161 ± 5 Å and in the birds 182 ± 5 Å. In the mammalian and amphibian species the dimensions were in agreement w th earlier work (185 ± 5 Å and 171 ± 5 Å, respectively)

Diffraction patterns from brachial plexus nerves of sheep fetuses were obtained from a gestation age of approximately 90 days. Weak lines indicating a lower number of regularly arranged radial repeating units compared to older fetuses wer obtained from fetuses between 90 and 105 days gestation age. The diffraction patterns from fetuses older than pproximately 105 days gestation age were similar to those from peripheral nerves of adult sheep

The first low angle x-ray diffraction studies of nerve myelin were reported by SCHMITT BEAR and CLARK (1935) and SCHMITT BEAR and PALMER (1941) They found that the radial repeating unit in nerves from various mammals and from reptiles (turtle) was about 184 Å, and that of amphibians about 1 1 Å. The relative intensities of the various orders of diffraction lines were, in order of intensity the second, fourth and third.

Several studies have been performed to determine the structure of the myelin sheath by means of x-ray diffraction (SCHMITT BEAR and PALMER 1941 FINEAN 1953) and electron microscopy (SJÖSTRAND 1953 FINEAN and FERNANDEZ MORÁN 1957) On the basis of X-ray diffraction data and electron microscopic

Table 1. Dimension of radial repeating unit in various vertebrate species

Species	No.	Size of radial repeating unit	Range
Homo	5	185 ± 3 Å	
Monkey	1	185 ± 3 Å	
Sheep	5	185 ± 3 Å	
Rabbit	1	185 ± 3 Å	
Guinea pig	1	185 ± 3 Å	
Mammals, average		185 Å	
Dove	5	182 ± 3 Å	181–183
Duck	3	181 ± 3 Å	180–182
Hen	5	183 ± 3 Å	182–184
Birds, average		182 Å	
Frog	3	171 ± 3 Å	
Toad	2	171 ± 3 Å	
Amphibians, average		171 Å	
Bream	6	161 ± 3 Å	
Mackerell	5	161 ± 3 Å	
Pike	2	161 ± 3 Å	
Salmon	3	161 ± 3 Å	
Trench	1	161 ± 3 Å	
Fishes, average		161 Å	

findings, FINEAN (1953) and later other authors (ROBERTSON 1958, STOECKENIUS 1959, WOLMAN and HESTRIN LAMER 1960) have proposed models for the molecular structure of the radial repeating unit of peripheral nerve. From electronmicroscopic observations GEREN (1954) found that the repeating units of the myelin sheath are derived from the Schwann cell surface by an infolding process initiated when the Schwann cell envelopes the axon.

In this study the size of the radial repeating unit was measured on peripheral nerves from various species of fishes and birds, and also on mammalian and amphibian species. In connection with investigations on the prenatal functional development of the nervous system (BERNHARD, KAISER and KOLMODIN 1959) diffraction patterns were also obtained from peripheral nerves of fetal sheep. A preliminary report was given at the X. Scandinavian Congress for Physiology August 1960 (HOGLUND and RINGERTZ 1960).

Methods

Apparatus. Unfiltered copper radiation from a fine focus X-ray tube (Ehrenberg and Spear) was used for all the exposures. A semi-micro low-angle camera with two permanent collimating lead slits mounted on holders allowing lateral adjustments was built for this purpose. The sample-to-film distance was 60 mm. CEA X-ray film, textur X, was used.

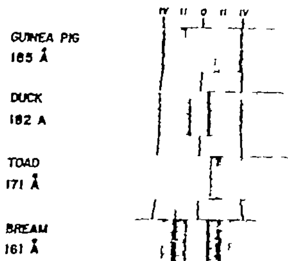


Fig. 1. Low-angle diffraction patterns recorded from peripheral nerves of mammal, bird, amphibian and fish. The centre of the primary beam (0) and the second (II) and fourth (IV) order lines are indicated. The obtained dimension of the radial repeating unit is given t the left.

A one millimeter deep brass chamber providing optimal thickness for diffraction of CuKα radiation by an organic compound was used. The chamber was bounded by two ten microns thick mica windows to provid a moist atmosphere within the chamber during the exposure. For the photometric recordings a Zeiss Schnellphotometer II was used.

Adult nerves The sciatic nerve was used from adult mammals, birds and amphibians and the cervico-brachial plexus from fishes. The animals were killed immediately before the nerve was excised The human sciatic nerve was taken from fresh autopsy material. The exposure time was 1/2—4 hours. The distances between the obtained lines on the developed film were measured under the microscope. The size of th radial repeating unit was calculated from the lines of the second, third and fourth order The error of measurement caused by the line width and the air scatter was about 0.03 mm, corresponding to $\pm$ 3 Å.

Fetal nerves Nerves from the brachial plexus of 22 sheep fetuses weighing from 21 to 4,600 g were used. The age of the fetuses varied from approximately 56 days, calculated from the formula by HUGGETT and WIDDAS (1951) to maturity at 150 days. The exposure time on nerves from fetuses was 2 to 20 hours

When nerve material from the same adult animal or fetus was repeatedly examined using varying exposure times, th diffraction pattern was found to be the same if the nerve had not been allowed to dry or exposed to greater changes in the temperature (ELKES and FINEAN 1953, FINEAN 1957

Results

Radial repeating unit dimension in adult nerve

In Table I are listed the sizes of the radial repeating unit from peripheral nerves of 15 species belonging to four vertebrate classes. In the table the error of measurement, $\pm$ 3 Å, is given after the obtained dimension. Diffraction patterns from species belonging to these classes are shown in Fig. 1

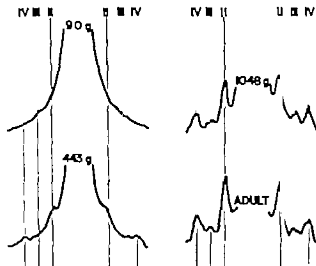


Fig. 2. Photometric recordings of low-angle diffraction patterns from brachial plexus nerves of sheep fetuses.

In the fishes (teleostei) investigated, the radial dimension of the repeating unit was 161 Å, and in the birds 182 Å. The radial dimension in the mammalian peripheral nerve, including that of man, was 185 Å, which is in agreement with the measurements by SCHMITT, BEAR and PALMER (1941) on other mammals. The values for toad and frog, 171 Å, was the same as found earlier. As seen in Table I, mammals, amphibians and fishes showed no variation between different species and animals of the same species. In the birds a slight variation between species and between animals of the same species was found, amounting to $\pm$ 1 Å each.

The relative order of intensity between lines of different order was the same for birds as for mammals and amphibians, namely second, fourth and third, while in the fishes it was found to be second, third and fourth.

X-ray diffraction patterns from fetal sheep nerve

Photometric recordings of diffraction patterns from brachial plexus nerves of fetal sheep of various gestation age are shown in Fig. 2. No diffraction lines were obtained from fetuses smaller than 443 g (gestation age approx. 90 days). The axons were thus surrounded by an insufficient number of repeating units for the recording of diffraction lines.

The diffractograms from fetuses between 443 g and 1 048 g (gestation age approximately 90 to 105 days) showed weak lines indicating a lower number of regularly arranged repeating units compared to older fetuses. In fetuses weighing 1,048 g or more, distinct diffraction lines of the second, third and fourth orders were obtained. The second order lines were the most intense

followed by the fourth and third. These diffraction patterns were thus similar to those obtained from adult peripheral nerve. The dimension of the radial repeating unit in the fetal nerves from which diffraction patterns were obtained was about the same as in adult mammalian nerve

Discussion

The differences in the dimension of the radial repeating unit between the birds and mammals was about 3 Å. This difference is smaller than the error of measurement and therefore can not be considered as significant.

At present no data are available showing variations larger than a few Ång strom units between different species within the same class of vertebrates but the existence of larger variations can not be excluded. Measurements on fishes, amphibians and birds have yet been reported for only a few species. Variations larger than a few Ångström units are therefore possible particularly within these classes. The relative order of intensity between lines of different order was not the same in the fishes as in the other investigated classes. This may be caused either by the dimension of the radial repeating unit or by a different structural arrangement within the unit.

Diffraction patterns from fetal nerves were obtained from fetuses of approximately 90 days gestation age. It may in this connection be noted that recent electrophysiological studies by BERNHARD KAISER and KOLMODIN (1959) have shown that the central nervous system of fetal sheep undergoes a rapid functional development during this prenatal period indicating a considerable increase of myelinated pathways.

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A Notice on Clinical Enzyme Unitage

Interpretation and translation of many arbitrary varied and unrelated *clinical enzyme units in current use has been a source of misunderstanding and* confusion among clinical chemists This will continue to be a vexing problem until steps are taken to reach some agreement on a common unitage. The problem is obviously complicated by crude reactants and poorly defined reactions. *It is, therefore, manifestly impossible to reach a completely satisfactory solution* in every case However it was the considered opinion of the Joint Sub-Commission on Clinical Enzyme Units of the International Union of Biochemistry and the International Union of Pure and Applied Chemistry meeting in Munich in 1959 that some clarification should be attempted, that dimensions should be in basic units, clearly defined and acceptable to all chemists and clinicians. The conclusion and recommendation of the Joint Sub-Commission was that wherever practicable clinical enzyme units should be defined as micro-moles of substrate transformed per minute under specified conditions, and their concentration in terms of a millilitre (or litre) of serum plasma or urine. Where this is impossible or difficult because of the complex nature of the substrate and uncertainty as to the nature of the products, e. g with fats, proteins or starch, the units should be expressed in terms of the analysable substances or groups determined to measure the reaction e g fatty acid, amino or carboxyl groups and reducing groups. Thus for instance, with a phosphate the enzyme activity can be expressed as micro-moles of organic phosphate hydrolysed (i. e. of phosphate liberated) per minute per millilitre (or litre) with amylase as micro-moles of reducing group

This recommendation is in conformance with the report of the Enzyme Commission of the International Union of Biochemistry

Monroe E Freeman Secretary *Sub-Commission (of IUPAC and IUB) on Clinical Enzyme Units* 1500 Massachusetts Avenue, N W Washington 5 D C U S.A.

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A Method for Continuous Measurement of the Carbon Dioxide Tension on the Cerebral Cortex

By

Bo K. Siesjö

Received 19 October 1960

Abstract

Siesjö, B. K. *A method for continuous measurement of the carbon dioxide tension on the cerebral cortex.* Acta physiol. scand. 1961. *51.* 297—313. — A method has been developed for direct, continuous measurement of the pCO_2 on the surface of the cerebral cortex, using a modification of the tissue pCO_2 electrode earlier described (Hertz and Siesjö 1959). The modification was made for the purpose of increasing the stability and accuracy of the measurements. The drift of the electrode was thereby reduced to less than 1 mV in five hours and the accuracy was increased to about ± 1.5 per cent. A reduction of the concavity of the pH glass membrane limited the response time of the pCO_2 electrode to 25—30 sec following a twofold increase in pCO_2. A description is given of the procedures for calibrating, suspending and thermostatting the electrode.

The validity and reproducibility of the pCO_2 values obtained when the electrode was applied to the cortical surface were investigated in cat experiments during respiratory and circulatory steady states. It was found that within a relatively wide range the pCO_2 was independent of the pressure exerted by the electrode on the cortical surface. Provided that the tissue did not present any swelling and that the tissue surface was not damaged or allowed to dry, the pCO_2 varied very little (coefficient of variation 1.5 per cent) when the electrode was repeatedly applied to the same or to different cortical areas. The influence of superficial cortical vessels was confined to the larger veins (diameter 0.5—1 mm), across which slightly higher (1—5 mm Hg) values were recorded. The findings are discussed in relation to the applicability of the method in studies of the acid-base metabolism of the tissue.

The importance of carbon dioxide for functional and circulatory processes in the brain (*cf* survey by MEYER 1958) has mainly been studied indirectly i. e. through the physiological effects induced by changes in the carbon dioxide content of the inspired air and the blood. The only direct study of the carbon dioxide exchange of the tissue has dealt with single analyses of the total carbon dioxide content of cortical tissue samples (BRODIE and WOODBURY 1958, NICHOLS 1958) In these studies, however it was possible to draw quantitative conclusions as to the acid base metabolism of the tissue only after assumptions had been made with regard to the pCO of the tissue, the solubility coefficient of carbon dioxide, and other factors. In no instance has a continuous measurement of any aspect of the carbon dioxide metabolism of the tissue proper been possible.

The development of a pCO_2 electrode for tissue measurements (HERTZ and SIESJÖ 1959) however provides certain possibilities in this respect (*cf* INGVAR, SIESJÖ and HERTZ 1959) The original electrode had the advantage of a suitable geometrical form, sensitivity and fastness in response. Yet a troublesome drift was a considerable drawback. Before the electrode could be made into a good instrument for quantitative investigations of the pCO of the tissue, its stability had to be improved as well as the accuracy of the measurements. In addition, factors which influence measurements on the surface of the tissue had to be analyzed.

The theoretical background for the measurement of the tissue pCO is th diffusibility of carbon dioxide and the potentiometric nature of the electrod principle (STOW BAER and RANDALL 1957) It follows that the electrode, if properly applied, should attain gaseous equilibrium with the tissue when placed on the surface of it. The value of pCO obtained on the arachnoidal membrane of a given cortical area cannot, however be directly used as an expression of the pCO of the tissue proper Thus, gradients of pCO must exist on the surface of the brain in relation to superficial arteries and veins The extent to which these vessels influence the pCO of different cortical spots obviously limits the importance of any quantitative conclusions f these are based on measurements on the surface. If so they are not valid for the cortical grey matter as a whole. Differences in pCO between different cortical area also tend to impair the value of such conclusions.

The aim of the present work is twofold In the first place it describes suitable modifications of the tissue pCO electrode so as to increase its stability as well as the accuracy of the measurements. Secondly it is intended as a methodol gical study of the problems encountered in the course of measurements on the surface of the feline cerebral cortex. The primary problem here was to investigate the optimal conditions for obtaining a minimum variation of pCO at a given cortical locus in a respiratory and circulatory steady state When such conditions were felt to be present, the variations of pCO encountered within the same or between different cortical areas were studied. Th

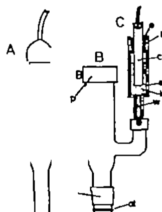


Fig. 1 The pCO_2 electrode unassembled. Only the calomel reference electrode shown in cross-section. *A.* shielded pH electrode. *B* glass housing with cover and screw of Perspex (p) for the fastening of the pH electrode rubber tubing (r) and o-ring (ot) to secure the Teflon membrane. *C.* calomel reference electrode with inner core ( ); Perspex housing ( ) o-rings ( ) t keep the core in the housing held apart by a Perspex tubing ( ) cotton wick (w) and potassium chloride (k)

relation between the pCO thus obtained and an a erage tissue pCO deter mined for the tissue proper will be dealt with elsewhere (GLEICHMANN *et al* 1961)

Material and methods

A. *The pCO electrode*

It seemed probable that the drift of the original electrode was, at least in part, due to base dissolved from the glass. It was therefore necessary to find kind of glass with especially low alkali solubility At the same time electrodes with more or less pronounced concavities of the glass membranes were tested. Another procedure tried for stabilization was the intercalation of special brand of paper (GIZEH-paper SCHOELLER and HOESCH, Gernsbach, Muretal) between the pH electrode and the Teflon membrane, as used in newly devised electrode for blood pCO measurements (GLEICHMANN and LUBBERS 1960)

The miniature *calomel reference electrode* earlier used (HERTZ and SIESJO 1959) had tendency to dry and could add to the drift of the pCO_2 electrode by means of changing diffusion potentials. An electrode with greater potassium chloride capacity was therefore used instead[a] (Fig 1) Contact between the potassium chloride solution (k) and the outer bicarbonate solution was made through cotton wick (w) In assembling the reference electrod small piece of cotton (w) soaked in the bicarbonate sol tion used, was packed into the lower end of the Perspex tube ( ) This was then half filled with saturated potassium chloride (k) The core ( ) with its o-rings ( ) was introd ced from above and held in position by the tight fitting of the o-rings (o) Excess potassium chloride was allowed t escape through small openings (not shown in the figure) in the upper part of the Perspex tube ( ) If silicone grease was applied to the Perspex around these openings, there was no need to refill the potassium chloride for several days.

As Perspex was found to dissolve appreciable amounts of carbon dioxide, *the electrode*

The generous help given by the firms INGOLD (Frankfurt am Main) and RADIOMETER (Copenhagen) is gratefully acknowledged.

[a] The inner core of the calomel reference electrode was kindly supplied by RADIOMETER

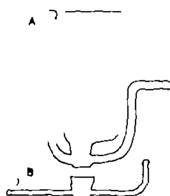


Fig 2 Calibration chamber in glass with inlet (I) and outlet ( ) *A* chamber for thermostatting the whole pCO_2 electrode. *B* chamber for *thermostatting the lower end* of the electrode.

housing (B) was made of glass with a cover of Perspex (p) for the fixation of the pH electrode (A) (Fig 1) The lower end of the housing had a groove for an o-ring (ot) which held the Teflon membran (6 or 12 micron) in position. A piece of rubber tubing ( ) around the lower end of th housing served to adjust the electrode in the calibration chamber As the pH electrodes later supplied (INGOLD Frankfurt am Main) were shielded, screening of the electrode housing was omitted.

In *assembling the electrode* the following procedure was followed sheet of the Teflon membrane (about 4 cm in diameter) was placed upon the o-ring and the lower end of the electrode was pressed against the o-ring until it slid onto the housing The o-ring was then transferred into the groove of the glass housing and the excess Teflon membrane bove the o-ring was wrapped around the rubber tubing, above which it was secured with rubber band. If instead the Teflon membrane was cut immediately above the o-ring, there might be electrical leaks in the course of the measurements on the tissue Next, the housing was filled with the sodium bicarbonate solution (0.001 or 0.0001 N) and t was carefully seen to t that no ai bubbles stuck to the Teflon membrane. At this stage the Teflon was tested for holes by gentle blowing at the end of the housing with th side branch closed. If there were any leaks in the membrane — even small ones — a small liquid drop was formed by the blowing. The pH electrode was then introduced into the housing and secured by means of the Perspex screw when the end of the electrode exerted slight pressur on the Teflon. Finally the calomel reference electrode was introduced into the sid branch of the electrod housing.

If GIZEH-paper was inserted between the pH electrode and the Teflon membrane a circular piece of the paper (diameter equalling the outer diameter of the electrode housing) was cut with a cork bore, thoroughly moistened with the bicarbonat sol tion and placed on the *Teflon membrane so that it covered the o-ring The same procedure* as described above was then followed.

The instrument used for the measurements was a pH meter (pH meter 22, RADIOMETER, Copenhagen) on which differences of 0.5 mV could be read conveniently The *ground* lead of the shielded wire of the pH electrode was connected t good ground, which also received a lead from the water bath (see below) The electrical stability was increased if some sodium chloride (about 1 per cent) was added to the water bath (*cf* Astrup 1959)

Calibration of the pCO_2 electrode was performed with streaming gas, which was preheated and saturated with water vapour in a water bath of constant temperature (± 0.1 C.) The gas slowly passed the calibration chamber which received the elec

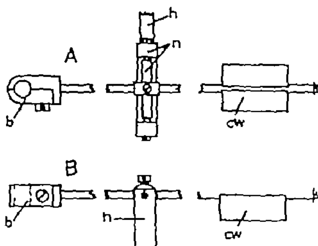


Fig. 3. Electrode holder, viewed from above (A) and from the side (B) with needle bearing ( ) bakelite clamp (b), counter weight (cw) and holder (h). The clamp is sized to fit the side branch of the electrode housing

trode (Fig. 2 A). Humidifiers, all rubber tubes, and the inlet to the calibration chamber were kept below the water surface. Unless these precautions were taken the value obtained for given gas concentration varied with the speed of the gas stream. Owing to the light sensitivity of the inner reference electrode of the pH lectrode, the latter was protected against direct sunlight during calibration.

The type of calibration chamber shown in Fig. 2 A made it possible to thermostat the whole pCO electrode in the water bath. To avoid convection in the air enclosed in the chamber cotton wool was loosely packed in the upper end around the electrode.

In *thermostating* the pCO_2 electrode for tissue measurements, any measure which appreciably increased the weight of the electrode had to be voided. The most satisfactory of the methods tried was the use of Perspex box around the head of the animal with holes for electrical leads, respiratory tubes, etc. Heated air was blown into the box by hair drier placed in funnel at the top of the box. The ir escaped through the holes. The temperature of the circulated air was adjusted to that of the cortical surface by change in the voltage across the heater of the drier. For this purpose the leads to the heat generating resistor were isolated and connected to ariable autotransformer. A syringe in the wall of the box and polyethylene catheter with its end lodged near the craniotomy made t possible to irrigat the surface of the brain with Ringer solution body temperature.

A simpler but less accurate way of obtaining temperature control was also tried. As before, the temperatur of the cortical surface was carefully measured with an electrothermometer based *upon the thermoelemen principle* (ELEKTROLABORATORIET Copenhagen) and the emperature of the w ter bath was adjusted accordingly. For calibration the chamber shown in Fig. 2 B was used. It was immersed into the water to depth where the Teflon membrane of the pCO electrode was just below the w er surface. The procedure ensured fairly identical temperature conditions for the pH glass and the enclosed bicarbona solution during calibration and measurement ($\pm$ 0.5° C. In both situations the calomel reference lectrode was exposed to room air the temperature of which could be kept reasonably constant ($\pm$ 0.5 C.) by means of thermostat (BRAUN Frankfurt am Main)

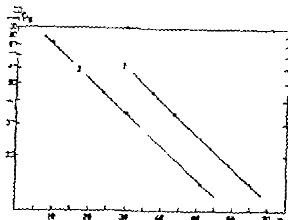


Fig 4 Calibration curve obtained on the day of assembling the pCO electrode (1) and after seven days (2), 37° C. and 0.0001 N NaHCO$_3$. Note parallel displacement of calibration curve and deviation from linearity at about 80 mm Hg

For the measurements on the surface of the cerebral cortex a mechanical *balancing device* was constructed, which minimized the pressure of the electrode on the tissue. The side branch of the electrode housing was fastened at one end of a lever which was suspended on needle bearing (Fig. 3) The weight of the electrode (30—35 grams) was balanced by a movable counter-weight. I this way the electrode readily followed small movements of the tissue synchronous with g the respiration.

B *The tissue measurements*

Experiments with observation of tissue pCO were mad on 24 cats. All animals wer anesthetized with Nembutal *(initial dose 40 mg per kg body weight p) In* cases where artificial respiration was employed curarizing agent (Flaxedil) was administered. The femoral ein was exposed and cannulated for injections and the femoral artery was treated in the same way for recording the blood pressure by means of an electro-manometer (ELEMA, Stockholm The skull bone was exposed through resection of the temporal *muscles* and one or *more holes were drilled in the bone, one of* which usually over the Suprasylvian gyrus. The dura was carefully split and the flaps were folded back over the bony edges The exposed cortical surface was protected from drying by intermittent irrigation with Ringer solution.

In most experiments the electrode was assembled with 0.001 N NaHCO nd with interposed *GIZEH-paper The electrode had weight of about 35 g. The lower end of the electrode housing had diameter of 8 5 mm Temperature control was obtained* by calibration at the same temperature as that of the cerebral ortex, and the larger part of the electrode was exposed to ambient air during both calibration and measurement. When the dura had been opened the electrode was mounted in u holder which was then placed close to the craniotomy so that the Teflon membrane of the electrode was parallel to the surfac of one of the gyri. The counter-weight of the lectrode holder was *then adjusted until the electrode was almost outbalanced and lowered so* that the electrode touched the tissue surfac Extreme car was taken so that the electrode readily followed the respiratory movements of the tissue without causing any dimpling of the tissue surface The thermojunction of the electro-thermometer was lodged beneath the dura t the border of the craniotomy The temperature *of* the water bath used for calibration was then adjusted to *that of the cortical surface* so as to permit *calibration immediately after series of measurements.*

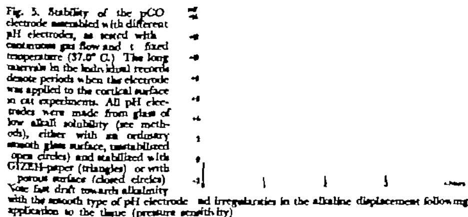


Fig. 5. Stability of the pCO electrode assembled with different pH electrodes, as tested with continuous gas flow and at fixed temperature (37.0° C.) The long intervals in the individual records denote periods when the electrode was applied to the cortical surface in cat experiments. All pH electrodes were made from glass of low alkali solubility (see methods), either with an ordinary smooth glass surface, unstabilized (open circles) and stabilized with GIZEH-paper (triangles) or with porous surface (closed circles). Note fast drift towards alkalinity with the smooth type of pH electrode and irregularities in the alkaline displacement following application to the tissue (pressure sensitivity)

Results

A. *Electrode characteristics*

The *sensitivity* of a pCO_2 electrode, which has been defined as $\frac{\Delta \text{pH}}{\Delta \log \text{pCO}}$ (SEVERINGHAUS and BRADLEY 1958) is influenced by the bicarbonate concentration and by the buffering capacity of any material used to keep a constant layer of reference solution between the glass membrane and the Teflon membrane. The actual mV sensitivity for a given change in pCO is, however also dependent upon the sensitivity of the pH electrode. Thus there was a danger of substantially reduced sensitivity in the case of the tissue pCO electrode, where special pH electrodes were used in connection with dilute bicarbonate solutions and where an interposed membrane was sometimes used for stabilization. It was found, however that the actual sensitivity of the electrode (1 pH unit = 61.5 mV at 37 C.) assembled with 0.0001 N $NaHCO$ and with interposed GIZEH paper was 0.88—0.90 (Fig 4) At higher bicarbonate concentrations and without the GIZEH-paper the sensitivity increased correspondingly (*cf* SEVERINGHAUS and BRADLEY 1958, GLEICHMANN and LÜBBERS 1960)

Since the sensitivity changes with hydrogen ion concentration (SEVERINGHAUS and BRADLEY 1958) the calibration curve could possibly be non-linear within physiological carbon dioxide concentrations. With the gas mixtures used in the present investigation (2 to 12 per cent carbon dioxide) no deviation from *linearity* could be detected using 0.001 N $NaHCO$ solution. With the 0.0001 N $NaHCO$ solution, however a small deviation from linearity could be seen at about 80 mm Hg (*cf* Fig 4) The dependence of the sensitivity upon the hydrogen ion concentration was noticeable when pCO electrodes with an alkali drift were used. Here the sensitivity was not constant but in-

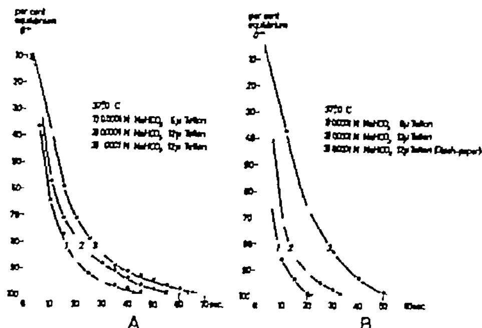


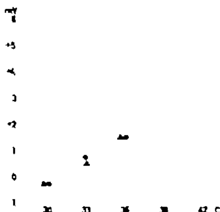
Fig 6. Response time of the pCO electrode obtained after twofold increase in pCO (usually 16 to 35 mm Hg) The curves were obtained with the porous type of pH electrode those in he right part (B) with relatively flat glass membrane

creased with th drift towards alkalinity i e the slope of the calibration curv changed with time

The *stability* of the pCO electrode is nfluenced by the characteristics of the pH electrode and by the stability of the calomel reference electrode Th latter was tested n a 0 001 N NaHCO solution against a commercial calomel reference electrode with an open liquid junction (K 100 RADIOMETER Copenhagen If the cotton wick of the reference electrode was previousl equilibrated w th the bicarbonate solution, the potential d'fference between the electrodes did not ex eed 2 mV any change usually being less than 1 mV in 12 hours.

The use of pH electrodes with glass membranes of low alkali solubility d d not prevent the drift of the pCO electrode previously experienced. As before the electrode displayed an initially fast, but later slower drift towards alkalinity (Fig 5 open circles) If, however GIZEH paper was used there was only an insignificant drift towards the acid side This latter drift did not usually ex eed 1 mV in 5 hours and was often less than 1 mV in 10 hours (Fig 5 triangles It did not matter if the GIZEH paper was allowed t cover the pH glass surface in the form of a sheet, or if it was inserted between the Teflon mem brane and the edge of the pH electrode in the form of a ring The acid drift of the pCO_2 electrod caused a slow parallel displacement of the cal br tion

Fig. 7 Temperature coefficient of the pCO_2 electrode as tested with continuous gas flow (6.23 per cent carbon dioxide). 0.0001 N $NaHCO$ and GIZEH-paper No correction made for the change in the water saturation of the gas. The values denoted by open circles and closed circles (different experiments) were obtained during heating those denoted by triangles during cooling in one of the above experiments (represented by the closed circles)



curve. This displacement, followed for periods of up to 7 days, was, however so small that no change in sensitivity could be detected (Fig 4)

One type of pH electrode tested (INGOLD Frankfurt am Main) made the pCO electrode sufficiently stable without the help of an inserted paper (Fig 5, closed circles) It was found that the surface of th glass membrane of this type of pH electrode was slightly porous, with a milky appearance. A change of this type extending over only a part of the glass membrane and the edge of the electrode was sufficient to render the assembled pCO electrode stable.

The *response time* of a pCO electrode depends, among other things, upon the bicarbonate concentration and on the thickness of the gas permeabl membrane (SEVERINGHAUS and BRADLEY 1958) The use of pH electrodes of the present type added another variable, the concavity of the glass membrane, in that a relatively flat membrane gave a faster pCO electrode. Fig 6 A shows the response time obtained after a twofold increase in pCO using the above-mentioned porous type of pH electrode with different bicarbonate concentrations and membrane thicknesses. If a pH electrode with a very flat glass membrane was included, the result was a fast pCO electrode. Thus, with 0.0001 N $NaHCO$ and 6 μ Teflon, 70 per cent equilibrium was reached after 6—7 seconds and 100 per cent after bout 25 seconds (Fig 6 B) In this case there was slight drift towards alkalinity Stabilization with GIZEH paper prolonged the response time so that changes were obtained which equalled those resulting from the ordinary type of electrode.

The *sensitivity to pressure* of the pCO electrode had to be taken into ccount when the electrode displayed an alkali drift. A sudden pressure on the Teflon membrane often gave a displacement of the calibration curve, mostly to the acid side This effect appeared to be greater the more the drift had changed the original pH value (*cf* Fig 5) Accordingly small effects or no effects

at all were seen when the present type of pCO_2 electrode was used. Repeated calibrations during the measurements on the brain also showed that the steady pressure of the electrode on the tissue even during large respiratory movements or vigorous pulsations did not influence the calibration curve.

The *temperature sensitivity* of the pCO_2 electrode was investigated with the type of calibration chamber shown in Fig. 2 A and with a continuous gas flow (6.23 per cent CO_2). A stable value after a change in temperature was not attained until after about one hour and the time of observation was usually prolonged to about one and a half hour at each temperature. Using 0.001 or 0.0001 N $NaHCO_3$ and GIZEH-paper small temperature effects were seen. Thus, in the region between 30 and 42° C. a change of 4° altered the reading by 1 mV in the alkaline direction during heating, in the acid during cooling (Fig. 7). This means that around 37° C. and at a pCO_2 of 45 mm Hg, a change in temperature of one degree changed the pCO_2 value obtained by 0.5 mm Hg.

The *accuracy* of the measurements as tested with repeated calibrations with the same gas mixture or repeated applications of the electrode to the calibration chamber was calculated according to the formula

$$\sigma^2 = \frac{\Sigma (X_{ij} - \bar{X}_j)^2}{N - j}$$

σ = Standard deviation
X_{ij} = measured value in mm Hg
$\bar{X}$ = mean of the j.th group
N = number of observations

All the groups (j) were supposed to belong to the same population. The results were transformed into a coefficient of variation (V) by applying the formula

$$V = \frac{100\,\sigma}{\text{mean}}$$

When calculated from 37 measurements in 8 groups, including measurements with the less accurate way of temperature control as well as measurements with intervals of a couple of hours, a coefficient of variation of 1.6 per cent was obtained.

B. *The tissue measurements*

The absolute pCO_2 as measured on the surface of the cerebral cortex of the anesthetized cat is influenced by several factors, the most important of which being changes in the pulmonary ventilation. As the chief aim of the tissue measurements in the present investigation was to determine the reproducibility of a pCO_2 value obtained in a given or in different cortical fields, the experiments were carried out in a steady state, *i.e.* the animals were usually ventilated artificially and kept in a moderate or deep barbiturate anesthesia. To exclude the possibility that the artificial respiration or the deep anesthesia was responsible for a stability that did not occur otherwise a few

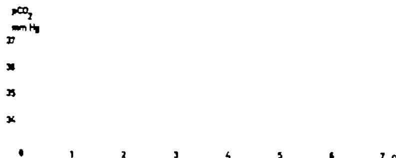


Fig. 8. Cat, Nembutal anesthesia and artificial respiration. Influence of the mechanical pressure of the electrode upon the value of pCO obtained on repeated application of the electrode to the same area (middle suprasylvian gyrus). Each circle denotes pCO at equilibrium (judged as constant reading for two minutes). The electrode pressure was increased by a change in position of the counterweight of the electrode holder. Diameter of the lower end of the pCO electrode 8.5 mm. At the pressure where the pCO started to increase (about 3 grams) the tissue surface was dimpled 0.5—1 mm.

experiments were carried out with lightly anesthetized, spontaneously breathing preparations.

After application of the electrode to the exposed arachnoidal surface of an arbitrarily chosen cortical area, an equilibrium was usually obtained after 3—6 min. If a steady state prevailed with regard to respiration, blood pressure and anesthesia, and if temperature fluctuations were avoided, the pCO thus obtained varied very little for periods of up to hours, and in many cases the variations during that time did not exceed a range of 2 mm Hg.

In the majority of the experiments the electrode was applied to a cortical area, which was exposed through a drilled hole of slightly greater diameter than that of the electrode. Since it was possible that carbon dioxide could be lost by diffusion from the surface, which might result in a false equilibrium, vaseline was in a few experiments applied around the electrode in the drilled hole. This, however, was found to cause no change in the pCO value previously recorded. Nor was there any increase in pCO if a gas mixture containing carbon dioxide at the same partial pressure as that measured was circulated around the lower end of the electrode.

The stability of the value of recorded pCO depended upon a good contact between the electrode and the tissue. If the pressure was too slight when the electrode was applied so that it intermittently lost its contact with the tissue surface, a lower and unstable value was obtained. The electrode could easily, however, be applied in such a way that it remained in contact with the tissue even during relatively large respiratory excursions without causing any dimpling of the tissue surface. If, then, an increased pressure was applied to the tissue by a change in the position of the counter-weight, the value obtained did not change significantly until the tissue surface was dimpled to a depth of about 1 mm. In these cases slightly higher values were recorded although the cali-

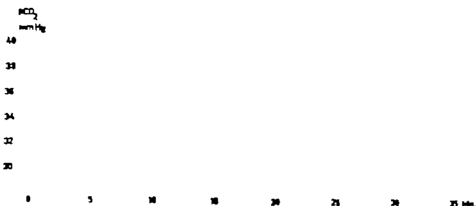


Fig 9 Cat, Nembutal anesthesia and artificial expiration. *Effect of vessel injury at the cortical* locus, where the pCO was repeatedly measured (middle suprasylvian gyrus) The open circles denote readings at 1 minute intervals in the course of two applications of the electrode. After 13 minutes three small superficial vessels were torn with a surgical needle, causing limited subarachnoidal bleedings. Note unstable and rising pCO (closed circles) after the vessel injury

bration curve of the electrode was not affected In one such experiment the pressure exerted by the electrode was measured in grams. It was then found that the pressure amounted to 3—4 g at the point where the pCO value began to increase (Fig 8)

The electrode holder made it possible to lift the electrode from the tissu surface and, after allowing time for partial equilibrium with air to reapply it to the same cortical locus, or to different loci within the same area. If this was done repeatedly the pCO value could usually be reproduced within a of 1 mm Hg The reproducibility thus tested did not change if the liquid on the cortical surface was carefully sucked away and if the surface was then irrigated with e g Ringer s solution, or if the tissue surface became polluted by blood from leaking bone vessels. This reproducibil ty was obtained on condition that there was no increased pressure in the brain with bulging in the bore hole and that the cortical surface was not allowed to dry In the latter case, due to exposure to air during longlasting experiments, a decreased reproducibility was often obtained as well as an increased susceptibility to the pressure of the electrode. A third factor of importance for the constancy of the pCO value obtained was an intact pial circulation. Thus, if the superficial pial vessels under the electrode were punctured or torn, unstable values resulted (Fig 9)

A large one-sided craniotomy with exposure of parts of two adjacent gyri made it possible to apply the electrode to a number of different cortical areas, avoiding direct application over the largest superficial vessels. A respiratory steady state could usually be upheld for periods of up to an hour allowing 5 to 9 measurements in a group The variations in the pCO values between the different seats of application were not greater than those found on repeated

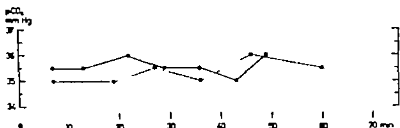


Fig. 10. Cat, Nembutal anesthesia, in one experiment with spontaneous (open circles) in the other with artificial respiration (closed circles). Reproducibility of value of pCO obtained on repeated application of the electrode to different cortical areas (Sylvian and suprasylvian gyrus). Constancy for two minutes judged as stable end value. In both experiments the values obtained varied within range of 1 mm Hg.

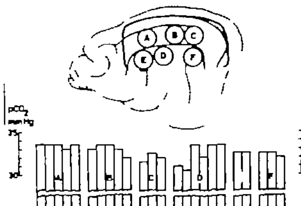


Fig. 11. Cat, Nembutal anesthesia and spontaneous respiration. Reproducibility of value of pCO obtained on repeated application of the electrode to different cortical spots (A—F) of an animal, which was kept in superficial anesthesia. Constancy for two minutes judged as stable end value. The individual measurements (in chronological order) denoted at the bottom of the figure. Occasionally during the experiment the animal was seen to move slightly with associated polypnoe (before measurements 5, 13 and 19). When this occurred additional anesthesia (2—3 mg Nembutal per kilo body weight) was given. Note small variations in the pCO values obtained (coefficient of variation 2.6 per cent) in spite of long observation time (about two and half hour) and correlation between the lowest values and the observed polypnoe.

application of the electrode to the same area, i.e. the values usually varied within a range of 1 mm Hg, irrespective of whether the animal was breathing spontaneously or was artificially ventilated (Fig. 10).

If all measurements with 5 or more determinations in a group (same or different spots) were put together and all experiments with observed changes in respiration and blood pressure during the time of measurement were discarded, 7 groups with 42 measurements were obtained. Statistical treatment based upon the assumption that all measurements belonged to the same population (see above) gave a coefficient of variation of 1.5 per cent.

Factors which tended to decrease the reproducibility of the pCO values obtained during a steady state were superficial anesthesia during spontaneous

respiration and prolongation of the time of measurement, an example of which is shown in Fig 11 It is seen that in this case there was a greater variation in the values obtained (coefficient of variation 2.6 per cent) The lowest values, however correlated pretty well with observed polypnoe.

The influence of pial vessels was investigated by comparing the pCO values obtained directly across superficial arteries and veins with those obtained in an area without such vessels. The pial arteries were found to have no significant influence upon the reproducibility whereas the values obtained over large veins (diameter 0.—1 mm) was 1—5 mm higher than elsewhere on the cortex. Although the pCO as obtained over different veins varied directly with the diameter of the vessel, the values measured over a given vein did not seem to vary more than in an area void of large veins.

Discussion

The pCO electrode The way of assembling the pCO_2 electrode must depend upon the type of experiment performed. Thus, the use of GIZEH paper has the advantage that pH electrodes with ordinary smooth glass surfaces can be used and that the concavity of the pH glass membrane can be reduced without risking unstability The porous type of electrode, however is preferable when rapid changes in pCO are to be recorded.

For ordinary use the 0.001 N NaHCO solution is recommended on account of the better linearity at higher carbon dioxide tensions than with other solutions. When a short response time is desirable the 0.0001 N solution should be chosen. In the latter case the use of 6 μ Teflon is a further advantage although the mechanical strength of th 12 μ membrane makes it better suited for most situations.

Two things are difficult to explain the cause of the reduced drift and the decreased sensitivity to pressure of the modified tissue pCO electrode. Th most probable explanation seems to be the following In the electrode arrangement the lower end of the pH electrode exerts a slight pressure on the Teflon membrane A small volume of bicarbonate solution between the Teflon and the pH glass membrane is thereby separated from the main bulk of the solution except for a narrow connection around the edge of the pH electrode The pCO electrode is sensitive to changes in the hydrogen ion concentration of the minor bicarbonate compartment. Such changes occur if carbon dioxid diffuses through the Teflon or if acid or basic substances are dissol ed from the pH glass. It is known that base is dissolved from all kinds of pH glass (cf KRATZ 1950) and this is thus the most probable explanation of the alkaline drift of the electrode The present work has shown that there were two ways of avoiding the drift, either by using pH electrodes with a porous glass surfac or by using stabilizing GIZEH paper This paper can be assumed to have a twofold effect, partly it acts as a buffer partly — by virtue of its pores — t allows substances to diffuse into the main bulk of th solution. The latter

effect seems to be the most important one since a stabilization was obtained also with the porous type of pH electrode It can thus be assumed that both procedures used for stabilization allowed the free diffusion of dissolved base from the minor bicarbonate compartment into the major one. Probably this exchange did not occur when there was a firm contact between the smooth edge of a pH electrode and the Teflon.

It seems that the sensitivity of the original pCO electrode to be the pressure applied to the Teflon membrane was due to variations in volume of the smaller bicarbonate compartment. Thus, an unequal distribution of base between the two compartments would be changed when pressure on the Teflon caused an exchange of solution between the compartments. Accordingly an electrode in which any base dissolved from the pH electrode is more uniformly distributed in the whole bicarbonate solution will be more resistant to pressure

The tissue measurements The cerebral cortex must be assumed to be subject to the same physico-chemical laws with regard to solubility and hydration of carbon dioxide and dissociation of carbonic acid as other systems in the organism. It follows from these laws that carbon dioxide being a gas, must exert a certain pressure at each point in the tissue and that this pressure has a certain local relation to the hydrogen ion concentration and the bicarbonate concentration. If the pCO changes these last mentioned variables change, and vice versa. Provided that the relations in the bicarbonate-carbonic acid buffer system of the tissue can be experimentally clarified, it is clear that the direct determination of one of the variables mentioned can give valuable information on the acid-base metabolism of the tissue proper

Owing to the lack of proper methods, information on the carbon dioxide metabolism of the cerebral cortex is scanty and mostly drawn from indirect studies. The only variable which has been studied directly is the total carbon dioxide content of samples of tissue removed from animals which had previously been allowed to breathe different concentrations of carbon dioxide (NICHOLS 1958, THOMPSON and BROWN 1960) This method necessitated the decapitation of the animal for a single analysis. It was possible that the pCO changed between the time of decapitation and the time of analysis. Besides the pH and the bicarbonate concentration of the tissue could only be calculated (BROOKS and WOODBURY 1958) after a series of assumptions concerning the actual tissue pCO_2, the solubility coefficient for carbon dioxide, and other factors.

It is obvious that a uniform pCO does not exist in the tissue where tension gradients are built up in the course of diffusion processes. It is convenient for practical reasons to express the different tensions of a gas in a tissue as an average tension (THEWS 1953, KETY 1960) Accordingly if useful in quantitative investigations, the pCO value obtained by a tissue pCO electrode should ideally either be identical with or have a constant relation to the average tension in the tissue proper

The tissue pCO_2 electrode obviously measures the pCO_2 that exists on the surface of the brain. This tension is not necessarily identical with the average tissue pCO_2 of the grey matter. If a uniform average pCO_2 is assumed for the tissue proper in a given cortical area, the first problem to be dealt with is the assessment of the constancy of the pCO_2 as measured on the pia-arachnoid membrane in that area. The pia-arachnoidea is not representative of the actively metabolizing tissue and further contains arteries and veins, which must have a marked influence on the pCO_2 in their neighbourhood. Thus, if placed directly across a vein, an electrode with a diameter of the same size as that of the vein would measure a value similar to the venous pCO_2. It is then clear that the ideal electrode is one that measures a mean value of all different tensions on the surface.

If the requirements for constancy and reproducibility in measuring the surface pCO_2 are satisfied, the second problem is to correlate the pCO_2 as measured on the surface of the brain with that of the cortical grey matter proper. A useful method for assessing the latter tension implies application of diffusion laws for calculating the average tissue tension (THEWS 1955, 1960, KETY 1957). This method requires information on the arterial and venous pCO_2 as well as on the metabolic production of carbon dioxide. For practical reasons, measurements of venous pCO_2 and cortical metabolic rate can only be made if venous blood, coming from different cortical areas, is used. This fact is bound up with the third problem, that of regional differences in pCO_2 between different cortical areas. It is clear that if such differences exist between regions which contribute to the common venous pool, the correlation between the tissue pCO_2 and the surface pCO_2 will be hard to establish.

The present work yielded certain information on the problems outlined above. First, the reproducibility obtained on the same cortical spot shows that a reasonably constant pCO_2 exists for relatively long periods in a given cortical area provided that the animal is kept in a respiratory and circulatory steady state and that certain experimental conditions are fulfilled. These conditions are moderate electrode pressure, a tissue with a moist and undamaged surface and without signs of swelling and, finally application of the electrode to an area which is void of large superficial veins. It is also indicated that the application of the electrode as such does not interfere with the measuring conditions. Second, the fact that the influence of the superficial vessels upon the value of pCO_2 obtained is confined to the largest veins reduces the importance of this influence since these veins can be easily avoided. An attempt to minimize the influence by an increase of the diameter of the electrode involves the drawback that the diameter becomes too great in relation to the size of the gyri. Third, the reproducibility of the pCO_2 values obtained in the investigated cortical areas indicates that, in the experimental conditions, the cerebral cortex can be looked upon as a rather uniform organ as far as the pCO_2 is concerned. It has previously been demonstrated that there are

regional differences in cortical blood flow in the cat (KETY 1960) which makes it probable that small differences in pCO_2 do in fact exist. Such differences, which may be so small that they escape detection with the present method, however are of minor importance for calculations pertaining to the acid-base metabolism of the tissue. The reproducibility found seems also to be sufficient for a correlation between the pCO_2 recorded on the surface of the brain and that existing in the tissue proper even if the determination of the latter involves measurements on cortical blood derived from a large cortical area.

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Aspects on the Glucose Metabolism of the Hypothalamus and the Pituitary in Goats

By

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Abstract

Andersson, B., S Larsson and F Pocchiari. *Aspects on the glucose metabolism of the hypothalamus and the pituitary in goats* Acta physiol scand 1961 *51* 314—324 — With the aid of C-labelled glucose certain aspects of glucose metabolism have been studied in different parts of the hypothalamus and in the posterior and anterior pituitary The metabolic pattern of the hypothalamus differs from that of the pituitary Also within the hypothalamus certain differences exist with regard t the fate of the glucose in the incubation medium. Large amounts of GABA are found in the hypothalamus and the posterior pituitary Glutamine was formed in all samples studied. I the anterior pituitary glutamine contributes about 25 of ll amino acids formed from the radioctiv glucose In the hypothalamus and the posterior pituitary bout 10 μg per 25 mg of tissue per hour of the glucose present in the incubation medium was converted into amino acids.
The findings are discussed in relation to previous biochemical and physiological studies on this subject.

Different parts of the hypothalamus have different levels of metabolic activities with respect to ³²P-turnover (Forssberg and Larsson 1954 1955) In a previous study of the fate of ¹⁴C-glucose different metabolic activities were observed in various parts of the rabbit brain (Chain et al 1960 a)

The present investigation was undertaken to study the fate of generally ¹⁴C-labelled glucose in particular parts of the hypothalamus of goats. The glucose metabolism of the posterior and anterior parts of the pituitary was also studied.

Abbreviation γ — aminobutyric acid GABA

Materials and methods

Generally labelled C-glucose was obtained from the Radiochemical Centr Amersham, England. The radioactive material was diluted t gi e a specific activity of 20 μC per mg

The composition of the medium wa as follows NaCl 0.098 M, KCl 0 027 M MgSO 0.001 M, KH_2PO 0.0004 M Na_2HPO 0.0175 M and radioactive glucose 0.0056 M — 3.6 mC/mM at pH 7.3 (CHAIN *et al* 1960 b)

Goats of different ages and sexes fed *ad libitum* wer killed by decapitation. The part of the brain and the pituitary to be studied were dissected out as quickl as possible The lapse of time when the animals were killed until the samples were placed in the Warburg vessels was bout 10 min. The tissue samples were weighed on a torsion balance and transferred to 0.5 ml of chilled incubation medium in small Warburg essels of about 4 ml total volume. The centre well of th vessels contained small rolls of filter paper soaked with 0 07 ml 30 % NaOH for the absorption of respiratory carbon dioxide

The following samples were taken (Fig 1)

1) The periventricular hypothalamic tissue adjacent to the third ventricl The sample included the paraventricular nucleus (1)

2) The ventromedial hypothalamic tissue ncluding the ventromedial hypothalamic nucleus (2)

3) Parts of the posterior pituitary (5) consisting mainly of neurohypophysial tissue but mixed to some degree with intermediate lobe tissue

4) Parts of the anterior pituitary

In some cases the mammillary bodies (3) nd the supraoptic region were also taken for incubation.

In all the experiments the tissue was aerobically incubated for 60 min at 37 C. The gas phase was pure oxygen and the oxygen consumption was measured every 15 mi The $^{14}CO_2$ was determined as described by VILLEE nd HASTINGS (1949)

After incubation, the medium was immediately transferred to paper for chromatography and the tissue was treated as described by CHAIN *et al* (1960) The solvents for the chromatography were also the same as those used by these authors. All hromatograms were scanned quantitativel by the automatic scanning device (CHAIN *et al* 1956, FRANK *et al.* 1959)

In some experiments the insolubl residue was treated with 0.5 N NaOH for 36 hours t 50° C, and transferred to platelets and counted. In the ther experiments this fraction was hydrolysed for 48 hours with 5 N HCl 100 C in sealed tube, the acid being removed by several re-suspensions in water and dried over KOH. The dried residue was dissolved in water and chromatographed bidimensionally as described by BELOFF CHAIN *et al.* (1959)

After scanning, the chromatograms from the hydrolysed insoluble residue were sprayed with ninhydrine in an acetone solution (0 1 w/v)

Results

The oxygen uptake of the different parts of the hypothalamus and of the posterior and anterior pituitary is shown in T ble I A and B Very small differences existed in oxygen uptake between the various parts of the diencephalon. The oxygen consumption of the posterior pituitary was slightly lower and the anterior pituitary had an oxygen consumption which was still lower than the hypothalamic areas. Table I also shows the ^{14}CO and C-lactate formatio

Table I A. Oxygen consumption, $^{14}CO_2$- and ^{14}C-lactic acid formation in different parts of the hypothalamus and the pituitary in goat

Oxygen consumption expressed as μl per 25 mg of tissue (wet wt.) per hour.
$^{14}CO_2$ and ^{14}C-lactic acid expressed as μg glucose converted per 25 mg of tissue per hour.
Number of experiments given in parenthesis. Mean values ± S. E.

	Periventr. tissue	Ventromed. tissue	Supraopt. tissue	Mammillary bodies	Posterior pituitary	Anterior pituitary
O_2	58.7 ± 3.1 (14)	54.0 ± 5.3 (10)	56.3 ± 2.7 (4)	55.2 ± 2.9 (5)	50.5 ± 3.2 (10)	26.1 ± 2.6 (9)
$^{14}CO_2$	11.9 ± 1.0 (14)	10.8 ± 1.9 (10)	9.9 ± 1.1 (5)	17.6 ± 2.1 (5)	8.7 ± 1.6 (10)	2.3 ± 0.3 (8)
^{14}C-lact. acid	102.3 ± 7.2 (13)	89.8 ± 4.3 (10)	100.4 ± 6.8 (5)	125.0 ± 10.2 (5)	72.3 ± 5.1 (10)	50.3 ± 1.3 (8)

Table I B. Oxygen consumption and $^{14}CO_2$ production expressed as μM per 25 mg of tissue (wet wt.) per hour

Tissue	μM O_2	μM CO_2	CO_2/O_2
Periventr.	1.73	0.40	0.23
Ventromed.	1.52	0.36	0.24
Supraopt.	1.62	0.33	0.20
Mammillary b.	1.57	0.59	0.37
Poster. pit.	1.35	0.29	0.21
Anter. pit.	1.17	0.08	0.07

These values largely followed the course observed for oxygen, except for the anterior pituitary where not only absolutely but also comparatively both these components were low. When regarding the "^{14}C" RQ:s (Table I B) that is the ratio between the CO_2 produced by the glucose in the medium and the total oxygen consumed there is very little difference between the various parts of the hypothalamus and the posterior pituitary. The anterior pituitary, however, had a "^{14}C" RQ which was very low — 0.07. The true RQ of the anterior pituitary was found to be about 0.85—0.90.

The quantities of the amino acids formed by the glucose in the incubation medium are shown in Table II and III. They amount to about 10 % of the glucose metabolized in the hypothalamus and in the hypophysis.

The total amount of amino acids formed was highest in the hypothalamus and in the posterior pituitary. The quantitative distribution of the amino acids formed in the hypothalamus showed certain differences (Table II). In each of

Table II. Amino acid formation from metabolised glucose in different parts of goat hypothalamus and in posterior and anterior pituitary

Results expressed as μg glucose converted per 23 mg of tissue (wet wt.) after 1 hour incubation at 37 C in O in 0.5 ml medium (glucose concentration 0.1 ; total radioactivity 10 μC per vessel) Mean values ± S. E.

Tissue	Alanine	Aspartic acid	Glutamic acid	GABA	Glutamine	Arginine	Residue	No. of experiments
PV	1.4 ± 0.1	0.9 ± 0.1	6.0 ± 0.7	3.5 ± 0.3	1.2 ± 0.3	0.9 ± 0.2	0.6 ± 0.1	14
VM	1.3 ± 0.3	0.5 ± 0.1	4.4 ± 0.6	2.6 ± 0.5	1.1 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	10
SO	1.3 ± 0.2	0.7 ± 0.1	3.8 ± 0.6	2.7 ± 0.4	0.8 ± 0.1	1.0 ± 0.1	0.6 ± 0.1	5
CM	1.2 ± 0.2	1.4 ± 0.2	6.1 ± 0.8	3.2 ± 0.4	1.1 ± 0.2	traces	0.2 ± 0.05	5
PP	0.7 ± 0.2	0.8 ± 0.1	4.1 ± 0.5	1.0 ± 0.03	0.7 ± 0.1	0.7 ± 0.2	1.1 ± 0.2	10
AP	0.9 ± 0.1	0.4 ± 0.05	0.7 ± 0.1		0.9 ± 0.2	0.4 ± 0.05	1.5 ± 0.2	8

PV = periventricular, VM = ventromedial, SO = supraoptic, CM = mammillary bodies, PO = posterior pituitary and AP = anterior pituitary tissue.

Table III. The percentage distribution of ^{14}C in amino acids formed by uniformly labelled glucose in different parts of the hypothalamus and the pituitary

The same abbreviations as in Table II are used

Tissue	Alanine	Aspartic acid	Glutamic acid	GABA	Glutamine	Arginine
PV	10.0	6.5	43.2	25.2	8.6	6.5
VM	12.6	4.9	42.7	25.2	10.7	3.9
SO	12.6	6.8	36.9	26.2	7.8	9.7
CM	9.2	10.8	46.9	24.6	8.5	
PP	8.8	10.0	51.2	12.4	8.8	8.8
AP	27.3	12.1	21.2		27.3	12.1

these parts and in the posterior pituitary more ^{14}C-glutamic acid was formed than any of the other amino acids. In the anterior pituitary the amounts of alanine, glutamic acid and glutamine were about equal. The formation of GABA was higher both absolutely and proportionally in the periventricular and ventromedial tissues (and in the supraoptic tissue) than in the pituitary samples (Table II and III) where only occasionally traces were found in the anterior pituitary

Glutamine formation from the glucose in the medium was observed in all the samples studied In the anterior pituitary it takes up a large proportion of the total aminoacids found to be labelled with ^{14}C (Table III)

A radioactive spot on the bidimensional chromatogram, identified as arginine,

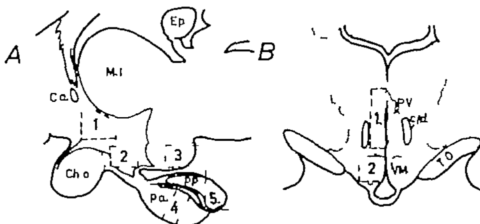


Fig. 1 *Diagrams of sagittal (A) and transverse (B) sections through the diencephalon of goat, indicating the areas taken for incubation.*
1 Periventricular tissue, 2 Ventromedial tissue, 3 Mammillary bodies, 4 Anterior pituitary 5. Posterior pituitary
Ep. = Epiphysis, M.I. = Massa intermedia, C.a. = Anterior commissure, p.a. and p.p. = anterior and posterior pituitary Ch.o = Optic chiasma, PV = Nucleus paraventricularis, VM = Nucleus ventromedialis, C.f.d. = Columna fornicis descendens, TO = Tractus opticus

was found in extracts from the various parts of the hypothalamus and the pituitary the periventricular and supraoptic tissues forming the greatest amount (0.9 and 1 0 μg) The percentage distribution of this substance is shown in Table III. One other still not identified spot on the chromatogram was found radioactive. It was mostly observed in extracts from the periventricular tissue After incubation of particularly the anterior pituitary but also the ventromedial parts of the hypothalamus small amounts of ^{14}C-proline was found

The total radioactivity of the insoluble residue was highest in the samples from the pituitary (Fig 1) In the hypothalamic samples studied, the periventricular tissue had the highest incorporation of ^{14}C in this fraction. Due to the relatively low radioactivity in the insoluble residue no strict quantitative data of the protein bound amino acids were obtained. Qualitatively however it seemed as if glutamic acid and alanine were radioactive in this fraction both in samples from the hypothalamus and from the pituitary

Using the technique mentioned previously radioactive glycogen and oligosaccharides were also found in the extracts from the hypothalamus and the pituitary Also in this case the anterior pituitary contained the greatest amount followed by the posterior pituitary

Discussion

In a recent study Cıᴀɪɴ *et al* (1960 a) found differences in the fate of radioactive glucose incubated with different parts of the rabbit brain, particularly in the distribution of certain amino acids. The hypothalamus divided into 3

samples were studied and also the cerebral and cerebellar cortici Enough tissue for incubation was not obtained if a further dissection was performed Instead, in the present study brains from goats were used since their larger size permitted dissection of discrete parts of the hypothalamus. The goat has also been used in several physiological studies of the hypothalamus (ANDERSSON et al 1958)

The values of the oxygen consumption of the hypothalamic samples agree well with those obtained by CHAIN et al. (1960 a) and largely with those found by SETCHELL (1959) on sheep brain pieces. The oxygen consumption was found to be highest in the periventricular and supraoptic tissue histologically these two parts of the hypothalamus have much in common. The explanation for the slightly higher oxygen values in these two tissues as compared to the ventromedial tissue remains unknown. However it has been found by histochemical studies that both the paraventricular and supraoptical nuclei contain more phosphatases than surrounding parts of the hypothalamus (ERÄNKÖ 1951)

Generally the amounts of CO and lactic acid formed by the glucose in the medium followed the oxygen consumption. This became more evident by regarding the "^{14}C" RQ s. The only exception to this was the anterior part of the pituitary where the "CO " RQ only was 0 07 ROBERTS and KELLER (1935) studied the oxygen consumption and lactic acid formation in hypothalamic and pituitary tissue before the administration of adrenaline or cortisone. They found a normal oxygen consumption which showed very small differences among the various parts studied In these experiments, however one has to consider the necessary inaccuracy involved in obtaining the tissue samples in the rat because of its small brain. In the present experiments small differences existed between the hypothalamic samples, but the anterior and also the posterior pituitary had a significantly lower oxygen consumption. As found by CHAIN et al (1960) small quantities of potassium will increase the oxygen consumption in the hypothalamus as well as in the cerebral and cerebellar cortici. This stimulating effect on brain slices was first observed by ASHFORD and DIXON (1935) and DICKENS and GREVILLE (1935) The values for the CO_2-formation from the glucose of the medium agreed well with those obtained from the hypothalamus in an earlier study (CHAIN et al. 1960 a) where it was shown that while potassium increased the oxygen consumption in the hypothalamus, the formation of CO from the medium-glucose diminished. In the cerebral cortex, however the CO formation was increased. It thus seemed that the addition of potassium partly increased the endogenous oxidation of the hypothalamus. In the present study as mentioned previously this effect seemed to be still more pronounced in the anterior pituitary where the "C" RQ was only 0.07 The true RQ of the hypothalamus was approximately 1 and even if the corresponding figure for the anterior pituitary was less (0 85—0.90) one can still state that the endogenous oxidation was very pronounced in this
The same tendency was met in the study by BELOFF-CHAIN et al. (1959)

the glucose metabolism in whole pituitary of rats had been observed. Here however the true values for the anterior pituitary were masked by the contamination of posterior pituitary tissue. Further CHAIN *et al.* used a phosphate saline buffer without potassium which could explain why their values on the oxidation of endogenous products were less than those reported here, where potassium was used in the buffer The mammillary bodies being an extrahypothalamic mesencephalic tissue had a "C" RQ which was highest of the tissues studied and resembled more the behaviour of the cerebral cortex in the study by CHAIN *et al.* (1960 a)

In the present experiments on the hypothalamus the labelling of the different amino acids from the glucose in the incubation medium followed the same gross pattern as that previously reported (CHAIN *et al.* 1960 a, TOWER 1959) Within the hypothalamus there were certain distribution differences (Table II and III) These were not big but they were consistent from experiment to experiment In the earlier studies on the rabbit hypothalamus such variations were not observed (CHAIN *et al* 1960 a) In the present study the observed differences in the distribution pattern among the amino acids probably depended on the further dissection of the hypothalamus. As was discussed by ANDERSSON *et al* (1958) various parts of the hypothalamus of the goat give different responses to electrical stimulation. Thus, the ventromedial and the periventricular parts of the hypothalamus have different physiological functions which might explain the differences in amino acid distribution. Regarding the earlier results by CHAIN *et al.* (1960 a) and the present ones it is evident that the brain is far from a biochemical unit. Such a conjecture is more justified in view of the earlier results showing that hunger and satiety caused profound differences in the levels of ^{32}P turnover and the formation of phosphorus containing high energy substances in small adjacent areas of the hypothalamus in the rat (FORSSBERG and LARSSON 1954 1955)

In all the hypothalamic tissues studied glutamic acid was formed in larger amounts than any other amino acid. This was in agreement with the earlier studies by CHAIN *et al* (1960 a) and by TOWER (1959) A discussion as to the central position of glutamic acid in the brain metabolism due to its ability to undergo oxidation, amidation, transamination and decarboxylation has been given by ANSELL and RICHTER (1954)

The present experiments have also confirmed earlier findings that the GABA formation of the hypothalamus is high (CHAIN *et al* 1960 a) Of the total amount of amino acids formed from the glucose in the medium, GABA took up about 25 % in the hypothalamic samples. The mammillary bodies, in spite of its close anatomical relation to the hypothalamus, only had a GABA formation which was about 15 % of the total amino acids formed. This amount was more related to that found in the cerebral cortex by CHAIN *et al* (1960 a) It is of interest to note that the posterior pituitary was able to convert glucose into GABA, while only occasionally traces of this amino acid could be found in the anterior

pituitary Since the discovery that the mammalian brain contains large amounts of GABA (ROBERTS *et al.* 1950 ROBERTS and FRANKEL 1950) this substance has received much attention. HAYASHI *et al* (1956) reported that GABA and especially γ-amino-β-hydroxy butyric acid inhibited convulsions induced in dogs by electrical or chemical stimulation The literature concerning the mode of action of GABA and its derivatives is still controversial (PURPURA *et al* 1959 CURTIS *et al.* 1959) The possible role of GABA or of related substances as a synaptic transmitter has not been proven by any direct experiments. However there seems to be little ground for doubt that this type of substances are of some importance for the brain. Certain results have indicated that the GABA content of the brain varies inversely with brain excitability (HÄKKINEN and KULONEN 1959) Preliminary experiments have also shown that certain tranquilizers selectively will influence the metabolic level of the hypothalamus, particularly the processes concerned with the GABA-formation (LARSSON 1960) The high rate of formation of GABA in the hypothalamus found earlier (CHAIN *et al.* 1960 a) and in the present study could partly be explained by the finding that the total amount of GABA is high in the diencephalon (ROBERTS and BAXTER 1959)

Glutamine is closely related to glutamic acid and GABA. While GABA is the decarboxylation product of glutamic acid, glutamine is the amid of glutamic acid. Considerable amounts of glutamine will be formed from the glucose in the incubation medium when potassium is present in the substrate (CHAIN *et al* 1960 a) Without potassium only traces of glutamine were formed in the hypothalamus while the cerebral and cerebellar cortices were still able to produce certain amounts of the substance (CHAIN *et al.* 1960 a) Also in the present study where exclusively a potassium containing medium was used, glutamine formation was high, not only in the hypothalamus but also in the pituitary As glutamine is the amide of glutamic acid one would assume that a high glutamic acid formation also should lead to a high glutamine formation, provided ammonia was available. The importance of accessability of ammonia for glutamine formation has been pointed out by TOWER (1959) In the present experiments it also seemed as the glutamine formation followed glutamic acid one except in the case of the anterior pituitary Here more glutamine is formed after incubation than glutamic acid, which could imply a larger excess to ammonia here BELOFF-CHAIN *et al* (1958) did not observe any glutamine formation in total pituitary from the rat, that is glucose in the medium converted into glutamine. In their experiments, however a potassium free medium was used, which will prevent or diminish the glutamine formation in the different parts of the brain (CHAIN *et al.* 1960 a) In the anterior pituitary the alanine production from the glucose in the medium was also found to be comparatively high, especially when regarding the percentage distribution of the amino acids. The high rate of formation of alanine and glutamine in the anterior pituitary in spite of its comparatively low "exogenous" activity totally might be explained

by the fact that these substances both are constituents of the active principles of the anterior pituitary (LI *et al.* 1958)

In the active peptide principles of posterior pituitary hormones, synthesised by hypothalamic neurones, glutamine is also present (DU VIGNEAUD 1955, BARTLETT *et al.* 1956). In the present study however no particularly high values for glutamine were found either in the hypothalamic or posterior pituitary samples. Slightly contradictory to the discussion above about glutamine as dependent upon the glutamic acid formation are the observations discussed by TOWER (1959) that the two substances not necessarily have to parallel each other under certain abnormal conditions of the brain. In the hydrolysed insoluble residue ^{14}C-glutamine was found in all the samples studied.

As mentioned previously ^{14}C-arginine was found after incubation with ^{14}C-glucose on the chromatograms from extracts of the two parts of the pituitary and from the periventricular and supraoptic regions, whereas this substance was not observed consistently in extracts from the other parts of the hypothalamus. In this connection it is of interest to note that RAFELSON *et al.* (1951) and WINZLER *et al.* (1952) have found that *in vitro*, essential and non-essential amino-acids incorporated ^{14}C from glucose to about the same extent in the brain. In the present experiments the appearance of radioactive arginine was encountered in the same areas that have neurosecretory activities. HOWE (1959) studied the distribution of arginine-positive material in the pituitary histochemically. In this study it seemed as if a histochemical reaction for arginine followed the location of neurosecretory material, and as mentioned above, in the present study the formation of arginine from glucose appeared to be located in the same areas.

The anterior pituitary seemed to have its own metabolic pattern and the most pronounced differences from the other tissues studied were the relatively high formation of radioactive alanine and glutamine. Further a comparatively high ^{14}C-activity in the "insoluble residue" was also found in this part. The amount of ^{14}C in the "insoluble" fraction was largely found to be in the protein-bound amino acids. Besides, the anterior pituitary high activities in this fraction were found in the posterior pituitary and in the periventricular and supraoptic parts of the hypothalamus. This might depend upon the special demands of these parts in building up hormonally active principles and neurosecretory material. Also the other parts of the hypothalamus studied contained some ^{14}C-activity in the protein fraction. This was in agreement with the studies by RAFELSON *et al.* (1951) and WINZLER *et al.* (1952). They found that when brain slices were incubated with radioactive glucose or the substance was injected into animals there was a rapid formation of ^{14}C-amino acids in the brain, both free and protein bound.

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The Effects of Moderate Sleep Deprivation on the Habituation of Autonomic Response Elements

By

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Abstract

SCHOLANDER, T. *The effects of moderate sleep deprivation on the habituation of autonomic response elements.* Acta physiol. scand. 1961. *51.* 325—342. — The phasic responses and the prestimulus values of the electrodermal activity, the pupillary size and the pulse rate, as well as the prestimulus respiration rate were recorded during monotonously repeated auditory stimulation. Intra-individual comparisons were made between the results obtained after one night of wakefulness and after normal sleep. Among the recorded variables only two, viz. the response amplitudes (i. e. maximal post-stimulus changes) of the electrodermal activity and the pupil were influenced in a statistically significant way by the sleep deprivation. The habituation of the response amplitude of the electrodermal activity was delayed and the mean level of the pupillary response amplitude was increased. It was assumed that these effects were due to a conflict between a wish to stay awake and the successively increasing sleepiness induced by the sleep deprivation and the monotony of the experimental situation.

Remarkably few physiological changes have been observed during moderate sleep deprivation. Reviews of previous research on this subject have been published by KLEITMAN (1939), TYLER, GOODMAN and ROTHMAN (19
and BJERNER (1949)

FREEMAN (1932) in studying performance found that the efficiency in different tasks could remain at a normal level for days following serious loss of sleep but that the muscular tension accompanying the work was higher under such circumstances. In an investigation which is specially relevant in the present context, BURCH and GREINER (1958) studied the electrodermal response (EDR) during a prolonged wakefulness of 30 hours. They included two different aspects of the EDR in their study. The "non-specific" response was measured "as the number of responses per unit time which occur due to internal stimuli, or to subliminal external stimuli not apparent to the observer". This nonspecific EDR showed a progressive depression with prolonged wakefulness. On the other hand the "specific" response (measured in per cent of the amplitude during a control period) to a mild electric shock either became depressed or increased successively during the experiment. The authors assumed that the depression of the specific response was due to "a condition approximating sleep" whereas the increase of the response reflected "the high amplitudes which they had observed during deep drowsiness and very light sleep". The distinction between "a condition approximating sleep" and "very light sleep" is unclear. However it is also stated, that the increase of the response amplitudes was obtained "in an active social situation with continued arousal and pressure for performance". This is important information which indicates that this response pattern might be a similar phenomenon to the above mentioned increase in the muscular tension obtained by FREEMAN (1932), viz. a reflection of the effort to maintain normal efficiency of performance in spite of the sleep loss.

The most consistent finding during sleep deprivation has been a decrease of the alpha activity of the EEG (KLEITMAN 1939, TYLER *et al.* 1947, BJERNER 1949, ARMINGTON and MITNICK 1959). Two different interpretations of this result have been proposed. TYLER *et al.* (1947) found that the decrease of the alpha activity was followed by an increase of the faster activity. They suggested that these changes might be caused by the effort in staying awake. On the other hand, BJERNER (1949) recorded EEG and pulse rate parallel to a serial reaction test. He showed that "delayed actions" in the reaction test were significantly associated with decrease of the number of alpha waves and of the pulse rate. Similar results were obtained by ARMINGTON and MIT-

The term used by the authors "galvanic skin response" (GSR), which is rather misleading (cf. WOODWORTH and SCHLOSBERG 19[illegible] p. 137). Because of this, and to make the description uniform, the more appropriate term "electrodermal response" (EDR) will be used in the following to describe acute changes in the skin resistance. The phenomena underlying the measurements of skin resistance and skin potentials will be called "electrodermal activity" (EDA).

Unfortunately the paper is only a [illegible] version of an article published under the auspices of N. A. T. O. The complete version has not been available. Consequently we lack certain important information about the experimental conditions, the number of stimuli used, the intervals between stimuli etc.

NICK (1959) during an auditory vigilance task. According to these authors the over-all decrease of the alpha activity is caused by sleepiness. They suggest that possible impairment of performance may be due to short periods of sleep or a condition like sleep. This "lapse" hypothesis is very attractive. Yet it seems somewhat inadequate to discard the interpretation presented by TYLER et al. (cf. WILLIAMS, LUBIN and GOODNOW 1959 p. 22). These authors emphasize that their subjects were highly motivated to stay awake. In addition the portions of the EEG records showing sleep activity were not used in the analysis. In this respect the experimental approach was different from that of BJERNER (1949) and of ARMINGTON and MITNICK (1959). Accordingly it seems probable that both the sleepiness and the effort to stay awake may decrease the alpha activity. The causal connection would be dependent on the experimental conditions.

Rationale and aim of the present investigation

In a previous investigation (SCHOLANDER 1960 b) it was shown that the habituation of EDRs and pulse rate responses was delayed when the subject was preoccupied with a simple sighting device. Ordinarily this device makes it possible for the subject to control his position in relation to a camera for recordings of the pupillary reactions. It was assumed that the delay of habituation obtained was due to increased alertness induced by the preoccupation with the sighting device.

In the present study the use of the pupillary camera could be expected to induce what might be called an "experimental conflict situation". It is a common observation that monotonous and prolonged repetition of a stimulus tends to induce a successively increasing drowsiness. It was assumed that this drowsiness would be more pronounced and appear earlier after a night without sleep than under ordinary circumstances. On the other hand, the above-mentioned results have indicated that the use of the pupillary camera counteracts the successive decrease of alertness consequent upon stimulus repetition. It is also obvious that the subjects' "anxiousness" to preserve good pupillary recordings would be a further incitement to stay awake.

It has been the aim of the present investigation to study the effects of this "conflict" between sleepiness and the wish to stay awake on the habituation of different autonomic response elements. The prestimulus values and the phasic responses of the electrodermal activity (EDA), the pupil and the pulse rate, as well as the prestimulus respiration rate were recorded during monotonously repeated auditory stimulation. Comparisons were made between the results obtained after one night of wakefulness and after normal sleep. In addition the pupillary recordings gave an opportunity to follow the tendency of the subjects to shut their eyes throughout the experiments. It was natural to assume that this tendency would be correlated to the degree of slee

It was predicted that after sleep deprivation the conflict between sleepiness and wish to stay awake would delay the habituation and increase the level of the phasic reactivity when compared with the results obtained after normal sleep Previous investigations have given few clues as to the possible effects on the prestimulus values. Consequently in this case no directional hypotheses were stated.

Methods

1 Laboratory and apparatus The laboratory and apparatus used in the present study have been described in detail elsewhere (DUREMAN 1959 SCHOLANDER 1960) The electrodermal activity (EDA) was recorded according to NICHOLS and DARROW (1955) In tabulating the EDA-data the conductance (micromhos) was used. A special camera which employs intermittent infrared light (DUREMAN, SCHOLANDER and SÄLDE 1959) recorded the pupillary reactions. The camera was coupled t the timer which initiated the sequence of the stimuli. I tabulating the pupillary data, square measure was used. This gave a better idea of phasic pupillary increments starting from large prestimulus pupils than linear measure (*cf* DUREMAN and SCHOLANDER 1959) In addition the pictures taken when the subject kept his eyes sh t could be counted. The film speed was 5 pictures per second and the number of pictures taken during each trial varied from 18 to 20 However uniformity was achieved by limiting the above-mentioned counting to the first 15 pictures of each trial.

The pulse and respiration rates were recorded by means of pneumographic devices (*cf* DUREMAN 1959 p 100) The prestimulus respiration rat was obtained by counting the number of cycles occurring within a 20 sec. interval preceding each stimulus. These values were multiplied by three to yield respiration rate per minut The somewhat cumbersome counting of the pulse waves was abandoned in favour of method for obtaining a direct, continuous recording (*f* SCHOLANDER 1960 a)

The stimulus, white noise signal with duration of 0 1 sec and an intensity of 105 db above threshold, was administered binaurally through headphones

2. Subjects The subjects were 12 students, 6 males and 6 females, aged 21 to 27 years. Each subject was present for 35 min on each of two days separated by three day interval. T control as far as possible the influence of d urnal variations each subject returned at the same time of the day

3. Procedure The subjects were divided at random into two groups of equal size In one group the first experiment was undertaken after one sleepless night (in the following called "no sleep condition) and the second experiment after a [illegible]ght with normal sleep (in the following called control" condition) In the other group the order of the sessions was reversed, first the control experiment and then the no sleep experiment. The subjects were given standard instructions. They were informed that the investigation was centred around the study of the influence of prolonged w ke-fulness on autonomic responses to repeated uditory timulation. They were [illegible]old that during the day and night before the control experiment they should follow their normal routine. They should sleep without any hypnotics and void stimulants, [illegible] coffee and tea on the morning of the experiment The subjects were told to keep wake during

I the following such pictures [illegible]ll be called closed-ey pictures. They were not only due to ordinary ey blinks. Especially after sleep deprivation the subjects tended to shut their eyes during longer periods and sometimes there was clear evidence tha these periods were due to sleep (*cf* p. 6)

the day and night before the other session but apart from this the instructions were exactly the same as for the control experiment.

It was further emphasized that continuous active cooperation on the part of the subject was necessary to guarantee optimal quality of the pupillary recordings. The subjects were instructed to maintain exactly the same position throughout the whole session by concentrating on the small red twinkling bulb which served as fixation point and which was seen through a simple sighting device on the camera. This part of the instructions was considered especially important in view of the results obtained in previous investigations (SCHOLANDER 1960 a, b).

Each session began with a period of 5 min during which the subject could relax and become accustomed to the apparatus. The flash tubes and the stroboscope of the pupillary camera make a faint clicking sound. To ensure habituation to this sound the apparatus was switched on and off 15 times with 5 to 10 sec intervals before 5 control exposures were made with the camera.

Immediately after this period the subject was given 30 "white noise" stimuli with intervals varying at random between 20 and 40 sec according to a sequence uniformly applied to all subjects during both sessions.

4 Treatment of data. All comparisons made in the present investigation were intra-individual. To simplify the treatment of data and to increase the reliability of the measurements, average values of successive blocks of 5 trials were computed. A distinction was made between 1) the so-called tonic activation or tonicity level, operationally defined as the prestimulus level and 2) the phasic reactivity. The latter was studied in terms of both the response amplitude, i.e. the maximal post-stimulus change, and the frequency of positive reactions during successive groups of five trials. For the comparison between the results obtained during the two sessions daily average values were used. As a gross measure of the change of the parameters within each session, individual "change indices", i.e. the average percentage divergences from the values at the beginning of the session were computed. First, the arithmetical mean prestimulus value or the mean response amplitude during the last 25 trials was expressed as per centage of the average value during the first 5 trials. Then, to get the percentage change, the obtained value was subtracted from 100 (cf. SCHOLANDER 1960).

Non-parametric statistical methods were used in the analysis of the data. To test possible changes occurring within sessions, Friedman two-way analyses of variance by ranks (SIEGEL 1956, p. 166 ff) were applied. The differences between the two sessions were tested by means of Wilcoxon matched-pairs signed-ranks tests (ibid., p. 75 ff). As a rule the null-hypothesis (H_0) was rejected in favour of the alternative hypothesis (H_1) if the probability yielded by the above-mentioned tests was equal to or less than $\alpha = 0.05$. A one-tailed test of significance was applied whenever H_1 indicated the predicted direction of the difference. When the results were against the predictions or no directional hypothesis had been stated a two-tailed test was used.

Results

The individual recordings

Before giving an account of the group trends obtained in the present investigation, certain interesting observations made during the individual recordings should be mentioned. In accordance with the results obtained in a

The observance of these instructions was not controlled but the subjects were relied upon to do their best. In any case possible divergences from the instructions would work against the given hypotheses and this possible source of error was therefore considered to be of minor importance.

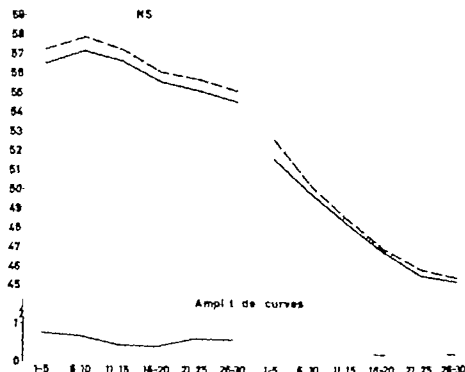


Fig 1 The mean alues of the prestimulus EDA (upper curves) and the EDR amplitude (lower curves) during successiv groups of five trials during the no sleep (NS) and the control (C) condition.

previous in estigation (SCHOLANDER 1960 a) the ndividual records of all variables showed considerable fluctuations. However this irregularity was more pronounced during the no sleep than during the control condition. The phasic response amplitudes of the EDA and the pupil in particular aried after sleep deprivation. High peaks with amplitudes two or three times as great as those obtained during the first trial often alternated with absence of response The fluctuations of the phasic response amplitudes showed a tenden cy towards increase during the latter stage of the no sleep experiment but this tendency was not uniform Moreover the above-mentioned high amplitude peaks appeared completely rregularly in the different functions. Thus a high EDR amplitude was seldom paralleled by a high pupillary response amplitude

In general the number of closed-eye p ctures increased considerably during the last 15 trials of th no sleep" experiment (cf p. 14) This increase was due mainly to the tendency of many subjects to keep their eyes closed during successively longer periods. In fact, 3 subjects under the no sleep" condition showed all external signs of sleep such as closed eyes, deep

Table Ia. Distribution of intra-individual differences in mean prestimulus EDA (in micromho) between the "no sleep" (NS) and the control (C) experiment

NS—C	Increase in conductance during C. > —40	—30	—20	—10	0	Decrease in conductance during C. 10	20	30	40	Mean diff.	T	p
n = 12	1				2	3	2	1	3	7 76	17	0 05

Table Ib. The sums of individual ranks computed from the mean prestimulus EDA during successive groups of 5 trials in the "no sleep" (NS) and the control (C) experiment. The results of Friedman analyses applied to these data

Trial	1—5	6—10	11—15	16—20	21—25	26—30	χ_r^2	Significance
NS	48 0	5[illegible].5	51.5	37.5	30.5	32.0	11.63	$p < 0.05$
C	64 0	51.5	40.5	41.0	29 0	26.0	23 87	$p < 0.001$

regular breathing and low muscular tension. During these sleep-like periods a successive decrease of the tonicity variables was seen. In addition the phasic reactivity usually decreased or disappeared but this finding was not constant.

Prestimulus EDA

In Fig 1 is shown a graph of the average EDA-data during the two experiments. In the upper curves the continuous lines represent the prestimulus levels and the broken lines the maximal post-stimulus levels. Consequently the response amplitude is the vertical distance between these lines. The amplitude data are further illustrated in the lower curves. Successive groups of 5 trials are plotted along the base line, the corresponding mean values of the skin conductance (in micromhos) and the conductance change along the vertical axis.

A comparison of the curves shows that the average prestimulus EDA stays at a higher level and declines less during the "no sleep" than during the control condition

To obtain a statistical estimate of these trends the individual average values of the prestimulus EDA during each of the two experimental sessions were first computed. Table I shows the distribution of the intra-individual differences between these average values. However when a Wilcoxon matched pairs signed-ranks test was applied a significant level was not reached ($T = 17$ $p > 0.05$ two-tailed)

To test the habituation tendency of the prestimulus EDA during each of the experiments the mean values to successive groups of 5 stimuli were computed and ranked intra-individually The sums of ranks (Table Ib) decrease con-

Table IIa. Distribution of intra-individual differences in mean EDR amplitude (in micromho) between the "no sleep" (NS) and the control (C) experiment

NS—C	Increase in mm² during C. —2.0	—1.5	—1.0	—0.5	0	0.5	1.0	1.5	2.0	2.5< Decrease in mm² during C.	Mean diff.	T	p
n = 12	1				1	2	2	4	2	1	0.09	13	< 0.025

Table IIb. The sums of individual ranks computed from the mean EDR amplitude during successive groups of 5 trials in the "no sleep" (NS) and the control (C) experiment. The results of Friedman analyses applied to these data

Trial	1—5	6—10	11—15	16—20	21—25	26—30	χ^2	Significance
NS	53.0	43.0	28.5	33.0	50.0	42.5	11.32	$p < 0.05$
C	65.5	55.0	41.5	40.0	39.5	30.5	17.72	$p < 0.01$

tinually during both sessions. When Friedman two-way analyses of variance by ranks were applied the control condition yielded the highest value of χ^2 ($\chi^2 = 23.87$, $p < 0.001$ versus $\chi^2 = 11.65$, $p < 0.05$).

Finally "change indices" (*cf* p. 5) were computed to make gross comparisons between the habituation trends possible. The intra-individual differences between the conditions in these indices yielded an insignificant value of T ($T = 19$, $p > 0.05$, two-tailed).

Thus neither the mean level nor the habituation of the prestimulus EDA showed significant differences between the experimental sessions.

EDR amplitude

A comparison of the curves derived from the average EDR amplitudes (lower part of Fig. 1) reveals that the control curve starts from a somewhat higher initial value than the corresponding "no sleep" curve. However, the former shows a clear-cut downward trend during the first 20 trials whereas the latter keeps at almost the same level throughout the experiment.

The amplitude data were treated in the same way as the prestimulus EDA. As predicted, a Wilcoxon test applied to the intra-individual differences between the two conditions in the average EDR amplitude (Table IIa) yielded a significant value of T ($T = 13$, $p < 0.05$, one-tailed).

The sums of ranks derived from the mean EDR amplitudes during successive groups of 5 trials are shown in Table IIb. These rank sums decrease during the first part of the "no sleep" experiment but show an upward trend during the last 15 trials. This tendency was so marked that a significant χ^2 value was

Table III. The sums of individual ranks computed from the frequency of positive EDRs during successive groups of 5 trials in the "no sleep" (NS) and the control (C) experiment. The results of Friedman analyses applied to these data

Trial:	1–5	6–10	11–15	16–20	21–25	26–30	χ^2_r	Significance
NS	60.0	45.0	24.0	52.5	43.5	47.0	18.44	p < 0.01
C	61.5	41.0	39.5	38.0	34.5	37.5	11.43	p 0.05

Table IVa. Distribution of between-individual differences in mean pupillary response amplitude (in mm²) between the "no sleep" (NS) and the control (C) experiment

NS–C	Increase in conductance during C.	Decrease in conductance during C.	Mean diff.	T	p
	< –0.8 –0.6 –0.4 –0.2 0 0.2 0.4 0.6 0.8 <				
= 11	1	1 1 3 2 2 1	0.49	13	< 0.05

Table IVb. The sums of individual ranks computed from the mean pupillary response amplitudes during successive groups of 5 trials in the "no sleep" (NS) and the control (C) experiment. The results of Friedman analyses applied to these data

Trial	1–5	6–10	11–15	16–20	21–25	26–30	χ^2	Significance
NS	46.0	41.0	34.0	38.5	46.5	46.0	3.09	
C	30.5	34.5	37.5	43.0	48.5	56.0	5.62	

obtained when the Friedman-test was applied ($\chi^2 = 11.52$, $p < 0.05$). During the control experiment the corresponding rank sums show a more successive decline which is highly significant ($\chi^2 = 17.72$, $p < 0.01$). When the difference in the habituation trend between the two sessions was tested by means of change indices a significant T-value was obtained ($T = 10$, $p < 0.025$ one-tailed).

It may be concluded that the "no sleep" condition induced a higher mean level and a less pronounced habituation of the EDR amplitude than the control condition.

Frequency of positive EDRs

The frequency of positive EDRs during successive groups of 5 trials was used as a complementary variable of the phasic reactivity. To make possible comparisons between the mean levels of this parameter the total number of

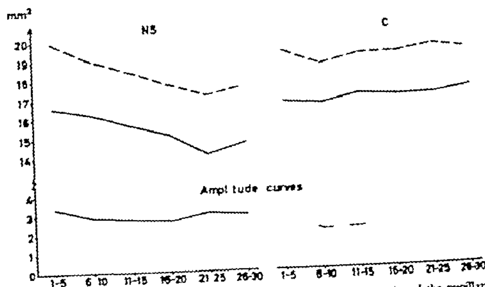


Fig. 2 The mean alues of the prestimulus pupillary area (upper curves) and the pupillary response amplitude lower curves during successn groups of fi trial during the no sleep" (NS and the control C cond tion

positive responses during each session was computed. The intra-individual differences of these values between sessions were however insignificant (T = 22, $p > 0.05$ one tailed)

The sums of ranks derived f om the frequency of positive EDRs during successive groups of 5 trials (Table III) show trends similar to those obtained for the EDR amplitudes. However the initial decrease and the subsequent increase of th rank sums during the no sleep" experiment is here ery pronounced and statistically highly significant ($\chi^2 = 18.44$ $p < 0.01$) During the control c ndition a fairly even habituation trend was obtained (χ^2 11 43 $p < 0.05$ Th ntra-indiv dual d fferences in the change indices" between sessions were not significant T = 24 5 $p > 0.05$ one tailed)

The frequency parameter d d not show any significant differences between the two sessions. H wever the initial decrease and subsequent increase of this variable as well as th EDR ampl tude during the no sleep condition is rather notew rthy Evidently th akefulness induced a marked tendency towards habituation during th first part f the experiment but this period was follo ed by a successiv l nc easing phase eactiv ty

Prestimulus pupillary are

In Fig 2, the pupillary data ha been shown in the same way as the EDA-data in Fig 1 All measurements are in mm The erage prestimulus pupillary area (upper curves) is somewhat smaller during the no sleep than during the control condition.

Further the "no sleep" curve shows a slight downward trend whereas the level of the control curve is relatively constant.

The intra-individual differences of the average prestimulus pupillary are between the two sessions did not reach a significant level (T = 20 p > 0.05, two-tailed). The sums of ranks derived from the mean values of the prestimulus pupillary area during successive groups of five trials revealed a decreasing trend during the "no sleep" condition and a slight increase during the control experiment. Neither of these trends were statistically significant ($\chi^2 = 10.01$ $p > 0.05$ and $\chi^2 = 4.91$ $p > 0.05$ respectively). According to the clearly significant value of T (T = 1., p < 0.05 two-tailed) obtained when a Wilcoxon test was applied to the corresponding intra-individual differences in the "change indices" the habituation of the prestimulus pupillary area appeared to be accentuated by the wakefulness. However it should be emphasized that a number of interpolations of the pupillary data had to be made during the no sleep condition due to the "closed-eye" pictures. No safe conclusions can therefore be derived from the analysis of the present variable.

Pupillary response amplitude

The curves of the average pupillary response amplitudes (lower curves in Fig 2) show differences which are in accordance with the predictions, i.e. a higher level and a less pronounced decline of the "no sleep" curve.

Among the intra-individual differences between the conditions in the pupillary response amplitude shown in Table IVa only two were against the predictions. A Wilcoxon test yielded a highly significant value of T (T = 13 p < 0.025 one tailed). Thus in spite of the fact that the number of zero values which had to be recorded due to the "closed-eye" pictures was greater during the "no sleep" than during the control condition (cf p. 14) the average pupillary response amplitude was still significantly higher during the "no sleep" experiment.

The habituation tendency was slightly more pronounced during the control experiment (Table IVb) but the intra-individual differences in the "change indices" did not reach a significant level (T = 36, p > 0.05 one-tailed).

Frequency of positive pupillary reactions

The statistical analysis of the frequency of positive pupillary reactions did not yield any significant intraindividual differences between the two sessions. A Wilcoxon test applied to the intra-individual differences in the total number of positive pupillary reactions yielded T = 21.5 (p > 0.05 one-tailed). Practically no habituation of the variable was obtained during the two conditions (during the "no sleep" experiment

It should be emphasized that during the "no sleep" condition the successively increasing number of "closed-eye" pictures made the pupillary recordings difficult. It was sometimes necessary to make interpolations between the prestimulus values obtained during the trials immediately before and after continuous series of "closed-eye" pictures, especially during the last ten trials. Whenever such periods made it impossible to obtain a value for the response amplitude zero was recorded. It is, of course questionable as to whether this was quite adequate. However with the pupillary response amplitudes the possible bias introduced by the procedure was against the predictions. Nevertheless this variable yielded results in agreement with the predictions.

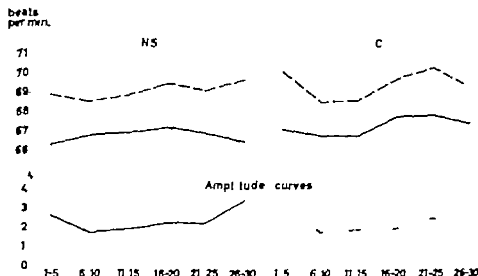


Fig 3 The mean alues of the prestimulus pulse rat (upper curves) and pulse ra response amplitude (lower curves) during successive groups of five trials during the no sleep (NS) and the control (C) condition

$\chi^2 = 1.92$ and during the control experiment $\chi = 0.30$) Consequently the tra-individual differences in the "change indices were also clearly insignificant ($T = 36$, $p > 0.05$, one-tailed)

Prestimulus pulse rate

A comparison between the curves derived from the average prestimulus pulse rat (Fig. 3 upper curves) reveals that the control curve keeps t a slightly higher level than the "no sleep" curve There is little or no tendency towards habituation. In fact the control curve shows even a slight upward trend.

A Wilcoxon test applied to the intra-individual differences in the a erage prestimulus pulse rate yielded $T = 32$ ($p > 0.05$ two-tailed) The habituation tendency tested by means of Friedman analyses also yielded clearly insignificant alues of χ^2 ($\chi^2 = 7.53$ during the "no sleep and $\chi = 5.06$ during the control condition) Finally the intra individual differences in the change indices" between the experiments were insignificant when a Wilcoxon test was applied ($T = 56$ $p > 0.05$ two-tailed)

Pulse rate response amplitude

The curves of the average response amplitudes of the pulse rat in Fig 3 hav certain common characteristics, viz. nearly equal levels during the first 25 trials. However during the last 5 trials the no sleep curve rises and the other falls. Thus only the control condition yields a certain tendency towards habituation of this ariable

As seen in the graph the intra-individual differences in the mean response amplitude of the pulse rate did not reach significant level ($T = 38$, $p > 0.05$, one-tailed) Furthermore, neither the no sleep nor the control condition yielded significa t degree of habituation ($\chi^2 = 7.33$, $p > 0.05$ and $\chi^2 = 6.29$ $p > 0.05$ respectively) When a Wilcoxon test was pplied to the intra-individual differences in the change indices" an insignificant T value was obtained ($T = 23$, $p > 0.05$, one-tailed)

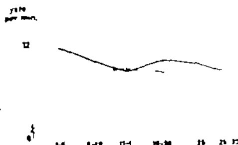


Fig. 4 The mean values of the prestimulus respiration rate during successive groups of five trials during the "no sleep" (NS) and the control (C) condition.

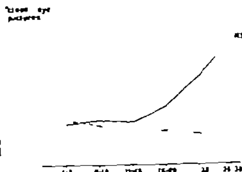


Fig. 5. The mean number of "closed-eye" pictures during successive groups of five trials during the "no sleep" (NS) and the control (C) condition.

Frequency of positive pulse rate responses

The statistical analysis of the frequency of positive pulse rate responses yielded results in good agreement with those obtained for the amplitude data, viz. no significant intra-individual differences in the total number of positive reactions ($T = 20$, $p > 0.05$, one-tailed) or in the "change indices" ($T = 26$, $p > 0.05$, one-tailed). The habituation tendency was also clearly insignificant during both conditions ($\chi^2 = 7.84$ during the "no sleep" experiment and $\chi^2 = 5.99$ during the control experiment).

Prestimulus respiration rate

It appears from Fig. 4 that the level of the average prestimulus respiration rate is somewhat higher during the "no sleep" than during the control condition. Both curves fall slightly and are almost parallel throughout the experiment indicating a tendency towards habituation.

When Wilcoxon tests were applied to the intra-individual differences between the two conditions in the average prestimulus respiration rate and the "change indices", insignificant values of T were obtained ($T = 22.5$, $p > 0.05$, and $T = 22$, $p > 0.05$ respectively). According to Friedman analyses the habituation of the variable was insignificant both during the "no sleep" ($\chi^2 = 5.26$, $p > 0.05$) and during the control experiment ($\chi^2 = 4.79$, $p > 0.05$).

Number of "closed-eye" pictures

In Fig. 5 the mean numbers of "closed-eye" pictures during successive groups of 5 trials have been plotted separately for each of the two experimental

Table Va. Distribution of intra-individual differences in mean number of "closed-eye" pictures between the "no sleep" (NS) and the control (C) experiment

NS—C	Increase in number of closed-eye" pictures during C. <—50	—40	—30	—20	—10	Decrease in number of closed-ey pictures during C. 0	10	20	30	40	50<	Mean diff.	T	p
n = 12		1	1				1	3	2	1	3	28.1	16	< 0.05

Table Vb. The sums of individual ranks computed from the numbers of "closed-eye" pictures during successive groups of 5 trials in the "no sleep" (NS) and the control (C) experiment. The results of Friedman analyses applied to these data

Trial	1—5	6—10	11—15	16—20	21—25	26—30	χ_r^2	Significance
NS	31.0	38.0	33.5	44.5	44.5	60.5	13.52	$p < 0.02$
C	53.5	42.5	38.0	43.0	41.5	33.5	5.18	—

sessions. The curves keep at approximately the same level during the first part of the experiments. However from the 15th trial onward the "no sleep" curve rises rapidly whereas the control curve falls.

b) The comparison between the total number of "closed-eye" pictures during the "no-sleep" and the control conditions was based upon intra-individual differences. The distribution of these differences is shown in Table Va. It appears that only two subjects had a greater number of "closed-eye" pictures during the "no-sleep" than during the control experiment. When a Wilcoxon test was applied a highly significant value of T was obtained ($T = 7$, $p < 0.025$ one tailed).

It was assumed (cf. p. 3) that the number of "closed-eye" pictures would be correlated with the degree of sleepiness. Thus it was natural to assume that the number of such pictures would increase during the "no sleep" experiment. To test this last assumption the number of "closed-eye" pictures during successive groups of 5 trials was recorded. The obtained values were ranked intra-individually. In Table Vb the sums of these ranks are shown. In agreement with the corresponding curve in Fig. 5 they increase during the "no-sleep" experiment. This increase was highly significant when tested by means of a Friedman analysis ($\chi^2 = 13.52$, $p < 0.02$). The corresponding decrease obtained during the control experiment did not reach a significant level ($\chi^2 = 5.18$, $p > 0.05$).

Discussion

Among the variables recorded in the present investigation only the response amplitudes of EDA and pupil were influenced significantly by sleep deprivation. Both these variables showed a higher mean level during the "no sleep" than during the control condition. However the most important change in EDR amplitude was the delay of habituation obtained after sleep loss. This conclusion can be drawn because marked intra-individual differences were obtained between the mean values of all trials whereas the corresponding differences between the mean values of the first 5 trials were clearly insignificant (*cf* the corresponding curves in Fig 1) It seems that the habituation of the pupillary response amplitude was not affected by the sleep deprivation. On the other hand it should be emphasized that the successively increasing number of zero values due to "closed-eye" pictures made a safe evaluation of the habituation of this variable during the "no sleep" experiment practically impossible.

It is interesting to note that the sleep deprivation induced a pronounced decline of both EDR parameters during the first part of the experiment whereas an increase was seen during the last 15 trials. This rising trend coincided with a marked increase in the number of "closed-eye" pictures. As previously mentioned such pictures were recorded both during ordinary eye blinks and when the subjects kept their eyes shut during longer periods. Evidently these periods were sometimes due to sleep-like conditions. Three subjects showed all external signs of sleep during the last part of the "no sleep" experiment. Afterwards they admitted spontaneously that they had been sleeping. However most of the subjects only reported pronounced sleepiness and increasing difficulty in keeping awake during the "no sleep" experiment. Under such circumstances it was impossible to decide whether the increased number of "closed-eye" pictures was caused by periods of sleep or simply difficulty in keeping the eyes open for other reasons. In fact there is some evidence that discomfort was the most common explanation. Irritation of the eyes, for instance, was a usual complaint after sleep deprivation. Three subjects appeared to fall asleep during the "no sleep" experiment, and all showed some kind av response decrement during the sleep-like periods. This is a similar response pattern to the one observed by BURCH and GREINER (1958) during a condition "approximating sleep". Thus it is reasonable to assume that an increase of the number of "closed-eye" pictures due to periods of sleep would have been followed by a decrease of the response amplitudes. On the contrary in the present investigation most subjects showed an increase in the

All the intra-individual differences between the two sessions derived from the tonicity variables were insignificant A possible positive relationship between the response amplitudes and the corresponding prestimulus values (*cf* SCHOLANDER 1960 a, 1961 b) is therefore of no importance in the present context.

EDR amplitudes during the last 15 trials of the "no sleep" experiment. This increase was mainly due to high amplitude peaks resembling those observed by BURCH and GREINER (1958) during "deep drowsiness and very light sleep". Moreover the mean frequency of positive EDRs during successive groups of five trials also increased.

According to the premises, both the delayed habituation of the EDR amplitude and the increased mean level of the pupillary response amplitude after sleep deprivation might be explained by the conflict between a successively increasing sleepiness and a wish to stay awake. BURCH and GREINER (1958) obtained a progressive increase in the EDR amplitude during similar experimental conditions, i. e. lack of sleep combined with "continued pressure for performance". The authors related this response pattern to what they called a "cortical release". This concept is vague and misleading. LOWENSTEIN and LOEWENFELD (1952) found that *habituation of the pupillary light reflex during monotonously repeated light stimuli progressed in a peculiar rhythmical way. After an even decrement during the first 6 stimuli the response amplitudes began to alternate in a wave like manner. The amplitudes of these waves progressively increased. They were more pronounced if the subject* was tired at the beginning of the experiment. It was suggested that the monotonous repetition of the light stimulus induced sensations of fatigue which in a feed-back loop were counteracted by centrally evoked sympathetic impulses.

It has been pointed out that the brain stem reticular formation plays an important role in the habituation mechanisms (SHARPLESS and JASPER 1956, CASPERS, LERCHE and GRÜTER 1958). In a recent survey (SCHOLANDER 1961a) it was suggested that the course of a habituation process "is at any moment dependent on the sum of extraneous activating, versus inhibiting impulses streaming into the brain stem reticular formation from other centres in the central nervous system as well as from the different extero- and endoceptors". Thus *if a hypothesis might be put forward concerning the neurophysiological background of the high response amplitude peaks observed in the present investigation it could be as follows.* The successively increased sleepiness induced by the experimental situation activates feed back loops between the brain stem reticular formation and, on the one hand, higher nervous centers and, on the other hand, different peripheral sensory mechanisms. These impulses would delay habituation and the sleepiness induced by this process. It is somewhat puzzling to find that the response amplitude peaks seldom appeared simultaneously in the different variables (*cf.* p. 6). However it is always difficult to compare momentary reactions elicited in different effector systems by a specified stimulus (*cf.* DUREMAN 1959 p. 60, BENDRA 1959 p. 219, SCHOLANDER 1961a p. 15).

It should be emphasized that at least one critical experiment must be made before the interpretation inherent in the premises of the present investigation

can be definitely accepted. The results would probably have been quite different if the subjects had been completely relaxed without any pressure for performance" Under such circumstances the combination of the sleep deprivation and the monotony of the experimental situation might increase the tendency towards both sleep and habituation of the variables as opposed to the results obtained after normal sleep. However most experimental situations involve some pressure for performance It is suggested that the instructions are of critical importance for the effects of sleep deprivation on physiological variables. If no care is taken to promote either complete relaxation or efforts to stay awake some individuals would probably show increased reactivity and delayed habituation. Others would exhibit decreased reactivity and an increased tendency towards both habituation and sleep. Finally a third category would be expected to alternate between these response patterns. Inadequate instructions may be one of the reasons why many previous investigators have failed to find any clear physiological changes after sleep loss (cf reviews published by KLEITMAN (1939) TYLER, GOODMAN and ROTHMAN (1947) and BJERNER (1949)) Furthermore it is evident that monotony and prolonged experiments may potentiate the effects of sleep deprivation. It can be mentioned that among a wide variety of psychological tests WILKINSON (1958) found that only two were significantly impaired after a wakefulness of 26—30 hours. Both these tests involved a prolonged monotonous repetition of a simple performance task. It was also shown that the decline of efficiency could be counteracted by giving the subject information of his results. It was concluded that "the less predictable the sleep-less operator finds the task situation and the greater the penalty he suffers for failing to predict it accurately the less likely is his efficiency to fall below normal levels"

One question which also remains to be answered is whether the present result would have been similar if the period of sleep deprivation had been prolonged Under such circumstances the conflict between sleepiness and wish to stay awake ought to increase The effects upon the recorded variables would then be more pronounced. Obviously there are great inter and intra individual differences with regard to tolerance of lack of sleep. Above a critical limit the subjects would be unable to withstand the sleepiness induced by the present experimental situation. The effects upon the recorded variables might then be contrary to those obtained in the present study

It has been a common clinical observation that sleepiness and fatigue if counteracted by will may cause psychosomatic symptoms such as headache, gastro-intestinal complaints etc. Mostly such symptoms have been interpreted as being compensatory phenomena caused by the effort to maintain normal efficiency Remarkably few experimental data supporting this assumption have been published. The observations made by FREEMAN (1932) indicate that the efforts to keep awake may be accompanied by increased muscular tension. In the present study it has been shown that changes in autonomic response

elements may also occur However there is need of further experimentation in this field. Many problems such as those mentioned above remain to be further elucidated.

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Acta physiol. scand. 1961 51 343—347

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Percutaneous Puncture of the Radial Artery with a Multi-Purpose Teflon Catheter for Indwelling Use

By

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Received 1 December 1960

Abstract

Barr, P.-O. *Percutaneous puncture of the radial artery with multi purpose Teflon catheter for indwelling use* Acta physiol. scand 1961 51 343—347 — A Teflon catheter assembly is described, which was designed for percutaneous insertion and indwelling use in the radial artery and in other superficial blood vessels with calibers of a similar order of magnitude The advantages of the instrument, which ma also be used for percutaneous puncture of other superficial tissues and organs, are reported and discussed, especially w th regard to the preferential choice of the radial artery for arterial punctures.

Three different methods are currently applied for percutaneous insertion of soft catheters into blood essels or other tissues, all of them using the principle of substituting a catheter for the hypodermic needle, which is required for the penetration of skin and tissue (1) introduction of a catheter through the lumen of the needle (Fitzpatrick, Schnabel and Peterson 1949 Peirce 1951 Gidlund 1956) (2) simultaneous insertion of a catheter and the needle, the needle having previously been pushed into the catheter for support and its protruding tip being used for puncturing (Massa *et al.* 1950 Seldinger 1937) and (3) threading a catheter over a flexible leader the leader having previously been pushed into place through the lumen of th hypodermic needle (Seldinger 1953 Berneus *et al.* 1954)

While the radial rtery is in many instar ces the essel of choice for sampl of arterial blood and intravascular blood pressure measurements, ... puncturing of deeper arteries may cause dangerous hematoma, none of catheter combinations previously described have proved practical f

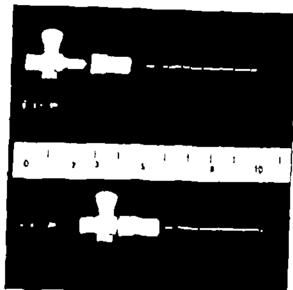


Fig 1. *Above* Elements of Teflon catheter *Below* The assembled instrument, ready for percutaneous introduction.
Scale in cm.

this vessel. However after extensive experimentation with plastic tubings of different kinds and dimensions it was found that by using the method of MAMA *et al.* (1950 see above) in combination with thin-walled tubing of Teflon, the radial artery may conveniently be catheterized with a minimum of trauma. While the instrument to be described was primarily designed for this artery it has proved equally useful for other superficial blood vessels.

Constructional Details

The instrument (Fig 1) consists of three main parts 1) the catheter 2) the cap and screw adapter with topcock, and 3) the puncturing hypodermic needle

1) The catheter is made of a 50—60 mm long piece of Teflon tubing[1] (inner diameter 0 80 mm, outer-diameter 1 05 mm) One end of the catheter is heat moulded into a flange the other end (tip of catheter) being cut off square and trimmed

2) The adapter similar to the metal cap and screw types that are commercially available for polyethylene tubing, is made of Teflon and incorporates a stopcock of the same material To prevent slipping of the catheter the V shaped seat inside the cap is reinforced by a metal bushing The flange of the catheter acting as its own gasket isolates the metal reinforcement from the lumen of the catheter-adapter

3) The puncturing needle is a standard hypodermic needle (short-bevel point) with a length of 80—100 mm and an outer-diameter of 0.8 mm.

[1] Tubing supplied by Hammarby Bakelit Industri AB, Stockholm, Sweden.

Technique for Percutaneous Insertion into the Radial Artery

The equipment is assembled by attaching the catheter to the adapter a d pushing the needle through the lumen of the catheter adapter The needle m at fit into the tubing so that it may be gently advanced under rotation The t p of the steel needle should protrude 2—3 mm out of the trimmed front end of t e catheter

Sterilization can preferably be done in the assembled state by boiling dry heating or autoclaving The instrument is inserted into the artery under infiltration anesthesia (for venous punctures anesthesia is not required In order to facilitate the localization of the radial artery the penetration of the skin and to reduce the tendency of spastic contractions of the vessel, it is recom mended to immerse the wrist and hand in water of 48—50 C for 5—10 min immediately before the insertion. Care should be taken that the anesthetic used is not too concentrated, since the hyperemia greatly increases the rate of absorption and the risk of toxic effects.

Since the diameter of the puncturing segment increases by only 0 25 mm at the tip of the Teflon catheter very slight extra pressure is needed after the protruding needle tip has been introduced. While the escape of blood from the needle indicates that the needle tip is inside the vessel, the slipping-in of the catheter tip is not always felt by the operator It is therefore recommended to advance the needle a few millimeters further into the vessel to make sure that the catheter tip has entered. Next, the needle is kept stationary while the catheter by applying pressure on the adapter is slid along the needle further into the vessel (BARR and SOLLA 1960) It is essential that the needle is not pulled out until the catheter is well in the vessel. On removal of the needle, blood spurts from the adapter and the stopcock may now be closed and reopened as needed

When removing the catheter the same precautions should be observed as when using hypodermic needles for arterial punctures. Thus, immediately after the withdrawal of the catheter local pressure should be applied first manually for 5—10 min, then by means of a pressure bandage for 3—4 hours (or more if the subject has been given anticoagulants) Excessive movements of the wrist should be avoided till the following day

Results

The needle Teflon catheter combination has been successfully used in this laboratory for almost 2 years in about 200 punctures. Of the insertions per formed, about 50 % were made into the radial artery and the rest on various peripheral arteries and veins. Of a total of over 75 subjects, most of them young healthy students, some have had the same radial artery punctured 8—12 times. Several subjects have had catheters in both radial arteries on the same occasion.

Since the opening cut in the artery is small and effective local pressure can easily be applied after removal of the catheter no serious complications have occurred In many instances arterial blood was drawn continuously from the catheter directly into a glass-reference-electrode assembly for continuous analysis of arterial blood pH For these measurements heparine had to be given intravenously in order to avoid clotting in the apparatus (100—150 mg Heparin Vitrum) In a few of these experiments, after which the subject had removed the bandage against instructions or vigorously moved his wrist before the heparine action had expired, moderate hematoma developed causing slight discomfort for 2—3 days. None of these subjects needed therapy Permanent after-effects have not been observed.

If handled with care, the same needle, catheter and adapter may after thorough cleaning, be re-assembled, sterilized, and used repeatedly However it is mandatory that the needle is free from the slightest barb and that the tip of the catheter is checked for accidental deformation

Discussion

The sampling of arterial blood is imperative in any physiological or clinical laboratory where investigations aim at complete evaluation of pulmonary gas exchange. Such sampling, as well as intraarterial pressure measurements, require arterial punctures when performed in humans. The present method was developed to eliminate some of the major difficulties and risks currently involved in this procedure Specifically a method was needed that could be safely employed for arterial puncture even in subjects treated with anticoagulants.

As to the site of the puncture, the radial artery was preferred mainly because the risks for accidental obstruction of blood supply to the hand are minimal, the collateral circulation being extensive (cf RADNER 1947) The ease with which effective pressure may be applied over the site of the puncture to counteract any tendency to hematoma is decisive for the choice of this artery when anticoagulants have been given to the subject.

There should be no need to emphasize the advantages of soft catheters for indwelling use in arterial punctures. However the methods designed for percutaneous insertion of flexible catheters into vessels (see above) were not found suitable for the radial artery either because the desired catheter lumen required a disproportionately big hole in the vessel wall or because the needle-catheter combination could not easily be heat-sterilized

While the instrument of MASSA *et al* (1950) exhibits the above-mentioned drawbacks, the method otherwise offers a most elegant solution to the problem. A search among various plastics (BARR and SOLLA 1960) showed that fluorocarbons might be used as catheter material to eliminate the disadvantages mentioned.

Mechanically Teflon tubing proved excellent for use with the relatively narrow radial artery since the thickness of the material could be reduced by more than 50 per cent, the tubing still retaining a sufficient strength to follow the needle tip through the skin, tissues and arterial wall without becoming deformed. The low coefficient of friction is another advantage of this material when used for introduction into tissues by the present procedure. Furthermore the tubing shows little tendency to kinking until the radius of bending becomes less than about 6 mm.

Chemically Teflon is the most inert plastic and the one least apt to cause tissue reaction (Le Veen and Barberio 1949 Harrison *et al* 1957) Since the wettability of Teflon is practically nil this plastic is less prone to induce clotting than most other materials.

Finally the thermal stability of Teflon allows sterilization of the entire catheter assembly by boiling or dry-heating up to 200° C, whereby infection due to imperfect sterilization by germicide, generally used for less heat-resistant plastics, can be avoided

Even if the present needle-Teflon catheter combination was primarily designed for the radial artery it has proved equally useful and convenient for other superficial arteries and veins, and should also be suitable for the percutaneous puncture of organs other than blood vessels.

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Acta physiol. scand. 1961 51 348—356

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Improved Technique for the Fluorimetric Estimation of Catecholamines

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Abstract

Euler, U S. v and F Lishajko. *Improved technique for the fluorimetric estimation of catecholamines.* Acta physiol. scand. 1961 *51* 348—356. — By the addition of small amounts of ethylene diamine (EDA) to th alkali-ascorbic acid mixture used in the trihydroxyindol (THI) method the discoloration of reaction mixture and instability of fluorescence can be prevented, allowing blanks to maintain their fluorescence values for several hours. The lutines obtained from adrenaline and noradrenaline standards and from alumina eluates are stable over period of t least one hour Optimal fluorescence values are obtained when the volume of alkali-ascorbic-acid — EDA exceeds the volume of the sample by 1 ml. A proportional increase in fluorescence is observed with sample volumes up to 3 ml. The technique used for estimation of catecholamines in urine and organ extracts is described. The importance of the acidity for the release of catecholamines from conjugates in urine t room temperature is emphasized.

Adrenaline and noradrenaline can be conveniently and accurately estimated in small amounts by fluorimetric technique using the trihydroxyindole (THI) method of Ehrlén (1948) and Lund (1949) When occurring in a mixture the two amines can be determined differentially after transformation to lutines, by the use of two filter sets (Price and Price 1957 Cohen and Goldenberg 1957 Euler and Lishajko 1959) or by using spectrophotofluorimetric technique (Bertler, Carlsson and Rosengren 1958 Vendsalu 1960).

Stabilization of the lutines is achieved by addition of ascorbic acid (Ehrlén 1948, Lund 1949) It is observed however that the mixture of ascorbic acid and strong alkali tends to age rapidly forming red-coloured compounds. This change is accompanied by an increase in the blank values and, under certain conditions, a decrease of the net fluorescence with time. For this reason

readings have to be made within a relatively short period after the reaction has been carried out, when extracts are used which have previously been purified by passage through an alumina column and eluted with acetic acid.

The present report deals with some observations made on the effect of addition of certain compounds on the stability of the reagent mixture and of the lutines formed from adrenaline and noradrenaline.

Experimental

1 *Stabilization of reagent blanks*

Spontaneous appearance of a reddish tint usually occurs in the alkali ascorbic acid mixture after some 7—10 min and develops further in the course of 1/2 hour During the same time, the initially slight fluorescence increases in the alkali-ascorbic acid mixture, rapidly during the first 5—10 min and then more slowly reaching a maximum in 15—30 min after which it remains unchanged over 4—6 hours. This increase in fluorescence is small for filter set A according to COHEN and GOLDENBERG (1957) consisting of primary interference filter 395—415 mμ and secondary filter Ilford Bright 623 peak transmission 490 mμ, but is considerable for filter set B, consisting of primary interference filter 436 mμ and secondary filter Corning 3486 with peak transmission 540 mμ. On addition of potassium ferricyanide the development of the fluorescence is markedly enhanced, particularly for filter set B.

It has been observed, however (F L.) that the increase in fluorescence is strongly inhibited by addition of small amounts of ethylene diamine (EDA) to the alkali-ascorbic acid mixture. No change of fluorescence with time was observed by addition of ferricyanide to alkali alone or to ascorbic acid alone Both ferricyanide and EDA caused, however a slight but constant increase in the blank figures.

By addition of ethylene diamine the development of the red colour in the alkali-ascorbic acid mixture was also postponed so that a slight reddish tint was observed only after 3—4 hours.

Effect of EDA on fluorescence of blanks with standard solutions of adrenaline and noradrenaline

Faded blanks were prepared by adding to 3 ml of the samples, containing 0.5 μg of adrenaline or noradrenaline, 0 1 ml 0.25 per cent potassium ferricyanide and 2 7 ml 20 per cent sodium hydroxide After 3 min 0.5 ml 2 per cent ascorbic acid was added.

Non-faded blanks were obtained by addition of 3 ml alkali-ascorbic acid mixture — 0 1 ml 0.25 per cent potassium ferricyanide to 3 ml of the sample containing 0.5 μg of adrenaline or noradrenaline standard respectively

Without addition of EDA the fluorescence of both the faded and the non-faded blanks with adrenaline, as well as noradrenaline increased, for filter set A by about 30 per cent and for filter set B by as much as 6 times in 60

Table I. Fluorescence (scale divisions) of blanks with standard solutions of A and NA. Faded and non-faded blanks with and without EDA

Standard	Blank	5 min		25 min		60 min	
		A	B	A	B	A	B
Noradr 0.5 μg	Faded	3.5	2.0	3.6	12	5.2	11.7
»	Faded + EDA	3.0	1.5	3.1	2.5	3.3	2.4
»	Non-faded	3.0	1.7	4.5	12	5.4	11.8
»	Non-faded + EDA	3.5	2.0	3.5	2.3	3.5	2.2
Adr 0.5 μg	Faded	3.0	2.0	3.9	13.7	5.3	12.4
	Faded + EDA	3.2	1.7	2.2	2.3	3.3	2.[illegible]
	Non-faded	3.2	[illegible].0	4.7	11.8	5.0	10.5
	Non-faded + EDA	3.8	3.4	3.9	4.0	4.0	4.0

When 0.2 ml EDA were added to 10 ml alkali-ascorbic acid mixture no increase in the sample blanks was observed over a period of 60 min (Table I).

Adrenaline and noradrenaline standard blanks prepared each hour over 4 hours with the same alkali-ascorbic acid mixture to which EDA had been added (2.5 : 100) showed no increase in the fluorescence values.

The minimum amounts of EDA required to prevent the development of the red colour in the reaction mixture, and to keep the reagent and sample blanks low, were found to be about 0.2 ml per 10 ml alkali-ascorbic acid mixture. Addition of 0.05—0.1 ml EDA to 10 ml had a marked although not complete protective action, while 0.01 ml was clearly insufficient. Since no advantage was observed by using higher amounts of EDA, which on the contrary tended to diminish the readings, the addition of 0.2 ml EDA per 10 ml alkali-ascorbic acid solution was used as standard procedure, being mixed with the alkali before addition of the ascorbic acid.

Effect of EDA on fluorescence of standard solutions of adrenaline and

The net fluorescence values of standard solutions of adrenaline after transformation to the corresponding lutines ascorbic acid mixture were well maintained for 1 hour with and (Table II) and thereafter declined slowly.

As seen in the table the net fluorescence values for markedly higher if transformation to lutines was made with acid to which EDA had been added. This occurred even if the acid solution containing EDA was 4 hours old. The net fl for adrenaline did not change however after addition of E 4 1/2 hours with the sample at room temperature and wi against day-light the decrease was less than 10 per cent. T decrease in fluorescence of about 5 per cent in 1 hour was o set B whether or not EDA was added.

Table II Blanks (Bl.) and net fluorescence (Net Fl.) of lutines from adrenaline (0 25 μg) and noradrenaline (0.5 μg) standard. Alkali-ascorbic acid mixture alone or with EDA prepared at time 0 Non-faded blanks. Filter sets A and B

		Adrenaline				Noradrenaline			
		Bl.	Net Fl.	+ EDA		Bl.	Net Fl.	+ EDA	
				Bl.	Net Fl.			Bl.	Net Fl.
5 min	A.	3.0	11.3	3 0	11.2	3.3	25.2	3 2	32 1
	B	4 8	33.2	2.2	33.3	4 0	24.6	3 4	29 1
60 min	A.	4 0	11.0	3.2	11 1	4 7	26.4	1.8	32
	B	10	31 4	2.3	31.5	8.9	25.2	2.2	28 6

The stabilizing effect of EDA was observed in the same way with 3 ml samples of a solution of 8 ml N HCl + 2 ml 1 M Na-acetate buffer pH 6 4 and containing 2 μg noradrenaline. The solution corresponds to the eluate obtained from ion exchange resins in the technique described by BERTLER *et al* (1958)

Eluates from urine and from organ extracts after adsorption on alumina

Normal urine was adsorbed on an alumina column and luted according to the technique of EULER and LISHAJKO (1959) Fig 1 shows the fluorescence values for blanks and the net values for samples with 3 ml eluate and 3 ml alkali-ascorbic acid, with and without EDA. It can be seen in the figure that the net fluorescence values are moderately increased after ddition of EDA, as in the case of noradrenaline standard. W thout the add tion of EDA the net fluorescence gradually declines over a 1 1/2 hour period, while no such decrease is observed after addition of EDA.

A number of tests were mad with alumina eluates from trichloracetic acid extracts of heart, spleen and liver of guinea pig In all instances the fluorescence figures in samples and blanks were constant over a period of 1 hour when EDA was added to th alkali-ascorbic acid mixture

Stabilizing effect of other compounds

The marked stabilizing effect of EDA on the development of fluorescence in the reagent mixture used in the THI method mad t ppear of interest to study the action of some related compounds in this respect The follo I g compounds were tested propylene diamine, pentamethylene diamine, meth yl amine, ethylamine, sodium sulphid hydroxylamine, gl tathion.

Propylendiamine added to a concentration of 20 mg/ml n the lkali-ascorbic acid mixture and hydroxylamine 0 1—0 3 mg/ml offered almost as good a protection as ethylenediamine in the same concentration Several of the other compounds also had a stabilizing ct on as shown by a dela

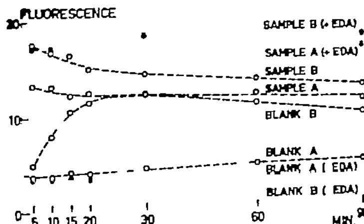


Fig. 1. Fluorescence values of blanks and samples from alumina eluates (urine) with and without addition of ethylene diamine (EDA) to the alkali-ascorbic acid. A and B denote filter sets. Fluorescence of samples are net values.

onset of coloration and maintenance of blank values. None of them appeared to offer advantages when compared with ethylene diamine, however.

Transformation of chromes to lutines

In a previous communication (EULER and LISHAJKO 1959) it was stated that the amount of alkali-ascorbic acid mixture which had to be added to the sample in order to obtain maximal formation of lutines depended on the volume of the samples. Thus it was recommended that for each ml of the sample 1 ml of the alkali-ascorbic acid should be added in order to obtain maximal fluorescence.

In the present study similar tests were performed, with EDA
alkali-ascorbic acid mixture.

Sample volumes of 0.5—5 ml of urine eluates were used and t
of alkali-ascorbic acid containing EDA for optimal fluorescenc
In order to reach maximal net fluorescence it was found that th
of alkali-ascorbic acid plus 1 ml was necessary as a rule in the
3 ml sample. With 4 ml of the sample the fluorescence was
less than proportional when compared with the results for 1
not possible to obtain an approximately proportionate fluores-
volume limit of 10 ml when 5 ml eluate was used. For this
mended that the volume of the eluate used should not –
proportionality of the fluorescence values was found up to this
2 ml sample of an alumina eluate from urine, the net
of the maximum with filter set A was 75 84 96 and 100
and 3 ml respectively of the alkali-ascorbic acid — EDA
With filter set B the corresponding figures were 70, 82, 97

The amount of ascorbic acid necessary to give optimal fluorescence was 1 ml of a 2 per cent solution in 9 ml 20 per cent Na OH to which 0.2 ml EDA had been added.

Oxidation time

Although 2 min were an adequate oxidation time for standards it was found that urine eluates required 3 min oxidation time in order to give optimal fluorescence values in several test series. Further increase in oxidation time did not increase the fluorescence figures.

Routine procedure for urine and organ extracts

To the filtered urine sample, usually 25 ml, 0.5 g of the disodium salt of ethylene diamine tetraacetic acid (EDTA) is added, and pH adjusted to 8.2—8.5 with N sodium hydroxide, added dropwise under continuous agitation with a magnetic stirrer and controlled by a glass electrode. The urine is then immediately passed through a column of 10 mm diameter containing 1 g aluminium oxide (British Drug Houses) The aluminium oxide is previously twirled around in distilled water and the resulting suspension of very fine particles, which may clog the glass filter of the column, decanted. This procedure is repeated 2 or 3 times or until the alumina settles rapidly leaving a clear aqueous layer

The 15 cm high glass tube is fitted with an upper wider part to hold 50—100 ml and a glass filter D1 and a glass stopcock in the lower part. The total passage time for the urine filtrate should not exceed 15 min. If passage tends to become slow air pressure can be applied to the column vessel so as to maintain a flow of at least 5 ml per min. Smaller volumes of urine than 25 ml are conveniently diluted to this volume with glass-distilled water After passage of the urine the glass walls and the column are thoroughly sprayed and washed with bi-distilled water until the washing fluid is completely free from alkali, which is essential.

Elution is performed with 0.25 N acetic acid of which 5 ml is thoroughly mixed with the alumina and allowed to filter off. Thereafter the same volume of acetic acid is added. The eluate is centrifuged at 600—1 000 g for a few minutes in order to sediment fine alumina particles that may have passed through the filter

In the eluate, which should have a pH value of about 3 5 and should not exceed pH 4 0, the catecholamines are stable at room temperature for several hours. When kept frozen, the eluate can be used for analyses weeks later Immediately before analysis, the eluate is adjusted to pH 6.2—6.3 with 2 N ammonia which is added dropwise under stirring with continuous control by the glass electrode. The eluate at pH 6.2—6.3 should be used for analysis as soon as possible in order to prevent spontaneous oxidation. Usually no losses are observed within 20 min, however

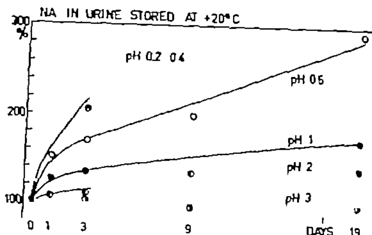


Fig. 2. Changes in free noradrenaline in urine kept at room temperature at pH 0.2—3 for various lengths of time. The curves represent different urines.

For larger volumes of urine a wider column may be used with a layer of alumina 1—2 cm high, allowing adsorption to be completed in 15 min. Elution is then made with acetic acid of sufficient volume and strength so as to maintain a pH value of 3.5—4 in the eluate.

Tissues and organs are minced and extracted with 2—5 volumes 10 per cent trichloroacetic acid for 1/2 hour filtered on a suction funnel and washed with 5 per cent trichloroacetic acid. The combined extracts are adjusted to pH 8.2—8.3 with NaOH.

To n ml of the sample, pH 6.2—6.3 in a graduated cylinder is added 0.1 ml 0.25 per cent potassium ferricyanide. Oxidation is carried out during 3 min. After this time n + 1 ml alkali-ascorbic acid containing 0.2 ml EDA per 10 ml is added and glass-distilled water filled up to 10 ml. After thorough mixing, the fluorescence is read in a fluorimeter with filter sets A and B. The Coleman 12 C fluorimeter has proven suitable for estimations in urine and most organ extracts. The blanks are prepared by adding alkali-ascorbic acid — EDA to the sample and then ferricyanide.

Influence of pH on free catecholamines in urine

It is well known that when urine is heated at acid reaction catecholamines are released from readily hydrolyzable conjugates. In order to ascertain the pH limit at which urine could be kept at room temperature without change in the proportion of free catecholamines, urines were adjusted to pH-values 0.2—3 and samples analyzed after various lengths of time. As seen in Fig. 2 considerable increases in free noradrenaline are observed at pH values below 1. At pH 3 the content of free catecholamines in urine shows insignificant changes over periods of more than 2 weeks. The results demonstrate the importance of maintaining an adequate reaction in urine stored before analysis.

Discussion

Although the trihydroxyindole (THI) method for the fluorimetric estimation of adrenaline and noradrenaline may be regarded as a simple and reasonably accurate method, the stability of the fluorescence in blanks and samples is often unsatisfactory. Thus the fluorescence of the blanks begins to increase in a few minutes, particularly when measured with filter set B according to COHEN and GOLDENBERG (1957). This does not invalidate the net fluorescence values of standards to any marked degree since these increase approximately by the same number of scale divisions. This is true also for an alkali-ascorbic acid mixture kept at room temperature for several hours. Since the blank values increase gradually for about 15 min the values may be inconvenient for the reading of low net fluorescence, however.

By the addition of a small amount of ethylenediamine to the alkali-ascorbic acid mixture it has been possible to stabilize fluorescence over a period of several hours. It thus becomes unnecessary to prepare new alkali-EDA-ascorbic acid mixture at frequent intervals. Care must be taken, however, to minimize the uptake of carbon dioxide from the air.

In addition to the advantage of maintaining low blank values another even more important advantage has been achieved. As mentioned above the fluorescence values for standard solutions show only little change over 1 hour but with samples prepared from urine, after adsorption on alumina and elution with acetic acid, the fluorescence shows a gradual decline, particularly of the values obtained with the filter β set. With the addition of EDA the fluorescence values obtained with such samples were constant for periods exceeding 1 hour. The moderate increase in fluorescence observed with filter sets A and B for noradrenaline is another advantage.

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Localization of Focal Potentials Evoked in the Red Nucleus and the Ventrolateral Nucleus of the Thalamus by Electrical Stimulation of the Cerebellar Nuclei

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Abstract

Appelberg, B. *Localization of focal potentials evoked in the red nucleus and in the ventrolateral nucleus of the thalamus by electrical stimulation of the cerebellar nuclei.* Acta physiol. scand. 1961 *51* 356—370. — The experiments described were performed on cats anesthetized with Nembutal. Electrical stimulation was applied to the cerebellar nuclei with stereotactically guided stimulating electrodes. Focal potentials evoked in the mesencephalon and in the thalamus were recorded with glass or tungsten microelectrodes. The potentials were histologically localized to the red nucleus and the ventrolateral thalamic nucleus. It was found that monosynaptic pathways exist between the interposite nucleus in the cerebellum and the red nucleus on one hand and between the interposite nucleus and the ventrolateral nucleus of the thalamus on the other. No activation of the investigated regions could be obtained by stimulation of the dentate or fastigial cerebellar nuclei.

It is well known that the red nucleus receives its main afferent supply from the contralateral deep cerebellar nuclei (Mussen 1927, Papez and Stotler 1940, Carrea and Mettler 1954, Carpenter and Stevens 1957). It is also known that some of the fibres of the brachium conjunctivum pass through the red nucleus without relaying. According to Allen (1924), Mussen (1927), Carrea and Mettler (1954) these fibres end in the ventrolateral nucleus of the thalamus. For many years the opinion seems to have been that both the interposite and the dentate nuclei are the cerebellar source for fibres to

the red nucleus. On the other hand JANSEN and JANSEN (1955) came to the conclusion that the dentate nucleus is mainly related to thalamocortical systems, while the interposite nucleus is associated with midbrain structures.

The present investigation was undertaken in order to reveal whether electrical stimulation in the cerebellar nuclei evoked a focal potential in the red nucleus. If this was the case, it would be the aim to find out if such a focal potential was localized to the red nucleus only thereby separating this structure from the rest of the mesencephalic reticular formation. This was regarded as being of importance for future electrophysiological work on the red nucleus and its relationships. During the course of the experiments it also became necessary to find the origin of the rubral fibres in the cerebellar nuclei and to localize a thalamic response evoked simultaneously with that observed in the red nucleus.

Materials and methods

The results presented were derived from 38 cats. They were anesthetized with Nembutal, the initial dose (25 mg/kg) administered intraperitoneally and repeated doses of 5—10 mg given intravenously when needed. The operation consisted in exposing the external meati acustici by sectioning the ears near the skull and in making trephine holes for the microelectrode to the mesencephalon and/or the thalamus, and for the stimulating electrode to the cerebellar nuclei. The animal was mounted in a Horsley-Clarke frame which carried the holder for the stimulating electrode. This holder was inclined 15° from the vertical plane, thereby orienting the stimulating electrode approximately in parallel to the bony tentorium.

The microelectrodes were guided with the aid of a micromanipulator of the type designed by G. J. Winsbury and described by ECCLES *et al.* (1954).

For stimulation in the cerebellar nuclei a mediolateral row of 5 steel needle electrodes insulated to about 0.4 mm from the tip and placed 1 mm apart was used. Stimulation was performed between pairs of nearby electrodes in the row. In some cases a single concentric electrode with an interpolar distance of about 0.5 mm was used instead. The site of stimulation was marked by electrocoagulation.

Recording was made with NaCl-filled glass pipettes, usually with rather crude tips (around 2—5 μ). These were able to record a focal potential which was not distorted by single unit activity. A chlorided silver plate screwed in bone over the frontal sinus was used as a reference.

In some experiments tungsten electrodes were used instead. These electrodes were prepared according to a method modified from HUBEL (1957). The tungsten wire (diameter 0.125 mm) was drawn into a glass tube with the microelectrode puller used for making the ordinary glass pipette electrodes. About 5 mm free tip of the wire was then sharpened electrolytically according to the Hubel technique down to a tip diameter around 1 μ. The whole electrode except the very tip was then covered by an insulating laquer (Voltalack 435, Standard Varnish Works Nordiska Aktiebolag, Goteborg) by lowering it tip upwards into a tube with thin laquer. Baking in 180° three times gave a thin but very resistant coating up to the tip.

These electrodes were well suited for recording from structures lying deep under the cortical surface as they could be made long and slender but still rigid thanks to the glass covering. They had the advantage that recording sites could be marked by means of electrolytic lesions. These lesions were made by passing a direct current of 5 μA for 10 sec through the tip of the electrode (tip negative).



Fig. 1 A. Focal potentials in the red nucleus evoked by electrical stimulation of the cerebellar nuclei. Time constant (T) 50 msec. Voltage calibration 100 µV Time 1 msec.

B. Same as A but slower sweep speed. T 0.5 sec. Time 10 msec.

For explanation of letters a, b and see text.

For microelectrode recordings an amplifier with a time constant which could be varied from 5 msec to 90 sec was used. As a rule time constants of 50 to 500 msec were used. Slow potentials of low amplitude could be checked by switching the amplifier over to DC recording The potentials were displayed on a double beam CRO and recorded on 35 mm film with a Grass camera. I all records negativity is recorded upwards.

A Grass stimulator (S4) and stimulus isolation unit (SIU4) were used for electrical stimulation. They were connected to a stimulus distribution unit which permitted rapid connection to any one of five output receptacles.

After each successful experiment the head of the animal was perfused with Ringer solution and with Bodian fluid. The brain was then embedded in celloidin. Sagittal or transverse serial sections, 50 or 100 µ thick were made through the cerebellum and through the mesencephalon and the thalamus. The sections were stained with toluidin blue (Nissl) As a rul the tracks mad with the stimulating electrode as well as th the microelectrodes could be seen and identified on the sections. Electrolytic lesions and electrocoagulations were always readily visible.

Results

I *Focal potentials evoked in the mesencephalon and in the thalamus by electrical stimulation of the cerebellar nuclei*

Electrical stimulation within the cerebellar nuclear complex evoked a characteristic focal potential in the mesencephalon in the region of the red nucleus. It consisted of an initial positive-negative spike (*a in Fig 1 A*) of short latency (mean latency to the maximum of the positivity 0.49 msec SD 0 09) This spike was followed by a negative wave (b in Fig 1 A) with a mean latency of 1 25 msec (SD 0.19) Its duration ranged between 2 and 6 msec. As a rule the potential complex ended with a longlasting positive deflection the duration of which varied between 50 and 100 msec (c in Fig 1 B).

If a synapse blocking agent (Myanesin) was administered intravenously while recording a response of the type described above the negative wave (b in Fig 1 A) and the shallow positivity (c in Fig 1 B) were seen to diminish and finally to disappear although the initial positive-negative spike (a in Fig 1 A) remained relatively undisturbed (as shown in Fig 2 C) A deepening of the anesthesia had a similar effect as is seen to the right in Fig — Also

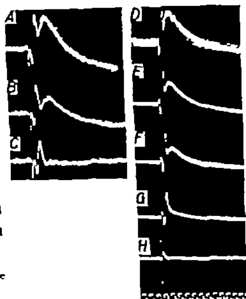


Fig. 2. To the left.

A. Control record of focal potentials in the red nucleus on cerebellar stimulation.
B. 2 min after i.v. injection of Myanesin (75 mg/kg).
C. About 30 sec later.

To the right:

D. Control record of potentials in the red nucleus.
E. After a total amount of 80 mg Nembutal given in 20 mg doses during 13 min.
F. After a total of 175 mg in 33 min.
G. After a total of 290 mg in 50 min.
H. Cat dead for about 10 min. Shows shape of stimulating artifact.

T 50 msec. Time 1 msec.

severe anoxia produced by stopping the artificial respiration in a curarized preparation made the negative wave disappear before the initial spike.

The type of response shown in Fig. 1 closely resembles that described by BROOKS and ECCLES (1947) and by ECCLES *et al.* (1954) for synapses in the spinal cord. It seems reasonable to interpret the different components of this focal potential in accordance with the above mentioned authors. Thus the initial positive-negative spike is attributed to the presynaptic volley and the slow negative wave is regarded as beeing of postsynaptic origin. Such an interpretation is supported by the results of Myanesin administration, increase in depth of anaesthesia, and anoxia.

The postsynaptic potential was found only within a very restricted area, while the presynaptic spike was more widely spread. When appearing alone, this spike was seen to be triphasic, positive-negative-positive. It was always considerably smaller in amplitude when recorded laterally, medially, dorsally and ventrally to the area giving a full response. Caudally to this area, between the red nucleus and the cerebellum, the presynaptic spike was big, and in a rostral direction it could be traced relatively unchanged up to the thalamus. There it was once more followed by a postsynaptic negative wave. Fig. 3 presents the shape of the presynaptic spike caudally and rostrally to the red nucleus (A and C) and also in this nucleus (B) and in the thalamic responding region (D). As shown in the figure the latency to the presynaptic spike increased in a rostral direction. The latency to the maximum of the initial positivity

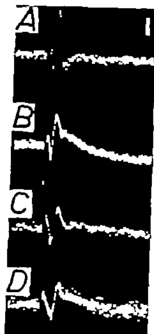


Fig 3. Response to cerebellar stimulation at the following frontal levels:

A. Caudally to the red nucleus. Latency to maximum of initial positivity 0.41 msec.
B In the red nucleus. Latency 0.50 msec.
C. Halfway between the red nucleus and the ventrolateral nucleus of the thalamus. Latency 0.63 msec.
D In the ventrolateral nucleus of the thalamus. Latency 0.85 msec.

Distance from the level of the cerebellar nuclei is to A about 7 mm, to B about 9 mm, to C about 11.3 mm and to D about 16 mm. T 50 msec. Voltage calibration 100 µV Time 1 msec.

was thus about 0.45 msec caudally to the red nucleus (Fig 3 A) and about 0 85 msec in the thalamus (Fig 3 D)

During the experimental work at the thalamic level it became evident that the latencies of the different components were not the same in the whole responding area. During penetration of a microelectrode through the thalamus a response sequence of the type illustrated in Fig 4 was regularly seen. Thus, the first sign of a response was a small, rather badly defined presynaptic potential followed by a post-synaptic negative wave with a latency of about 2.3 to 2.5 msec (Fig 4 A) As the microelectrode was pushed ventrally the postsynaptic potential suddenly diminished in amplitude while the presynaptic spike grew more visible (Fig 4 B) A little deeper the negative wave indicating synaptic activity once more increased. This wave, however now had a latency varying between 1 5 and 1 8 msec (Fig 4 C) This type of response was then seen through the rest of the responding part of the track. The latency difference of about 0.7 msec makes it probable that the dorsally recorded response is mediated from the cerebellum via a path with one extra synapse

II. *The localization of the focal potentials to morphological structures within the mesencephalon and the thalamus*

In these experiments the recording microelectrode was placed in a position which according to Horsley-Clarke coordinates ought to be the red nucleus or the ventrolateral nucleus of the thalamus. Then the stimulating electrode



Fig. 4. Response obtained from microelectrode in the ventrolateral nucleus of the thalamus. Electrical stimulation in the cerebellar nuclei.

A. First sign of evoked potential. Latency to postsynaptic wave (arrow) 2.50 msec.
B. 1 mm more ventrally. Weak response of the same type. Latency 2.40 msec.
C. 0.50 mm further ventrally. Strong response with latency to negative wave 1.60 msec.
D. Weak response of same type near the ventral border of response (3 mm ventrally to C).

T 50 msec. Voltage calibration 100 µV. Time 1 msec.

was guided to a position in the cerebellar nuclei where electrical stimulation evoked a good response from the macroelectrode. The stimulating electrode was kept at this point throughout the rest of the experiment. The stimulating intensity was usually between two and three times that evoking a threshold response.

Rows of microelectrode tracks 0.5 to 1.0 mm apart were then made through the responding regions. Usually only one or two such rows were made in one experiment. In each track photographic recordings were made at regular intervals (0.25 or 0.50 mm).

The tracks were then identified on serial sagittal or frontal histological sections. Tracings were made from enlarged sections with identified tracks. As a rule, all tracks in a grid were not found in one and the same section. A diagram was therefore made presenting all tracks on a tracing of a representative histological section. This procedure was generally satisfactory as the borders of the nuclei did not differ considerably between nearby sections. In some cases, however, an error was introduced. This is then commented upon in the text or indicated on the diagram.

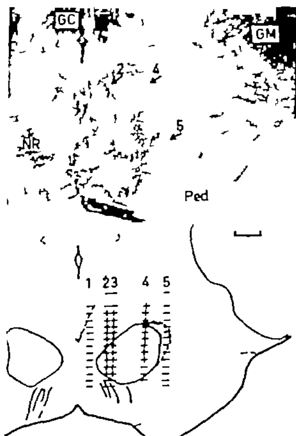


Fig. 5. Frontal section through the mesencephalon at the level of the red nucleus (top) and diagram of the same section (below). In this and the following figures faint traces of tracks are indicated by numbered arrows on the sections. Corresponding numbers are found in the diagrams. Plus symbols (+) on the diagrams indicate response to cerebellar stimulation and minus symbols (—) indicate lack of such response. GC, griseum centrale. GM, corpus geniculatum mediale. NR, nucleus ruber. Ped, pedunculus cerebri. Scale 1 mm.

The localization of the response in the mediolateral and rostrocaudal direction was established by the identification of the microelectrode tracks in the histological slide. The determination of a recording point along a track, i.e. the dorsoventral localization was, however, subject to some error introduced by the Horsley Clarke technique. It was due to the inaccuracy of using the earplug center as a reference point. Tungsten electrodes were utilized in order to determine the localization of a recording site in the dorsoventral direction. With these electrodes it was possible to make electrolytic lesions, thereby marking the site of the electrode at the moment it was recording a response.

Fig. 5 shows a frontal section through the mesencephalon at the level of the red nucleus. Four microelectrode tracks (marked by arrows and numbered 1, 2, 4 and 5) can be seen. On the diagram below, which is a tracing of the same section, the tracks are shown in their whole length. Track 3 in the diagram was identified on a nearby section. Plus symbols in the diagram indicate a response evoked by electrical stimulation in the cerebellar nuclei. Minus symbols indicate lack of such response. It becomes evident that the

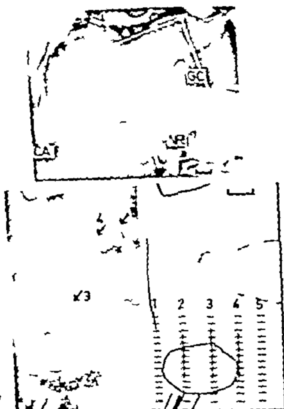


Fig. 6. Sagittal section through the brainstem about 3 mm laterally to the midline (top). The framed area is shown below to the left in higher magnification. Below to the right is diagram of the same area. GC, griseum centrale. NR, nucleus ruber. CA, commissura anterior. Scale 1 mm.

response was seen only in the tracks passing through the red nucleus and was not observed either medially or laterally to that structure. It should, however, be noted that track 5 in Fig. 5 did not show a response although it is actually seen to pass through the lateral part of the red nucleus. A careful examination of the section reveals that the lateral part of the nucleus here consists of a well defined pool of very small cells (the shaded area medial to the broken line). This is probably the so called nucleus minimus described by MAHAIM (1894) and by VON MONAKOW (1909).

The rostrocaudal extent of the mesencephalic response is shown in Fig. 6. In the upper part of this figure a sagittal section through the brain stem approximately 3 mm laterally to the midline is shown. The framed area is seen in higher magnification below to the left. Three tracks numbered 3, 4 and 5 are seen in the section. The diagram shows five tracks, of which tracks 1 and 2 were identified on nearby sections. Tracks passing rostrally and caudally to the red nucleus were negative. The ventral part of track 4, which appeared about 200 μ medial to the traced section, was seen to pass through the caudalmost cells of the red nucleus.

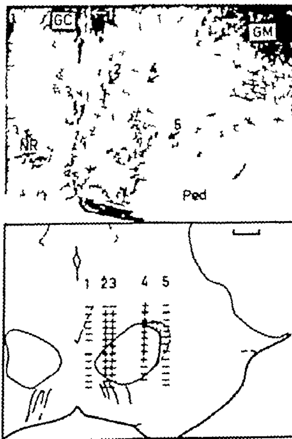


Fig 5. Frontal section through the mesencephalon at the level of the red nucleus (top) and diagram of the same section (below). In this and the following figures (last) traces of tracks are indicated by numbered arrows on the sections. Corresponding numbers are found in the diagrams. Plus symbols (+) on the diagrams indicate response to cerebellar stimulation and minus symbols (−) indicate lack of such response. GC, griseum centrale. GM, corpus geniculatum mediale. NR, nucleus ruber. Ped, pedunculus cerebri. Scale 1 mm.

The localization of the response in the mediolateral and rostrocaudal direction was established by the identification of the microelectrode tracks in the histological slide. The determination of a recording point along a track, i.e. the dorsoventral localization was, however subject to some error introduced by the Horsley-Clarke technique. It was due to the inaccuracy of using the earplug center as a reference point. Tungsten electrodes were utilized in order to determine the localization of a recording site in the dorsoventral direction. With these electrodes it was possible to make electrolytic lesions, thereby marking the site of the electrode at the moment it was recording a response.

Fig 5 shows a frontal section through the mesencephalon at the level of the red nucleus. Four microelectrode tracks (marked by arrows and numbered 1 2 4 and 5) can be seen. On the diagram below which is a tracing of the same section, the tracks are shown in their whole length. Track 3 in the diagram was identified on a nearby section. Plus symbols in the diagram indicate a response evoked by electrical stimulation in the cerebellar nuclei. Minus symbols indicate lack of such response. It becomes evident that the

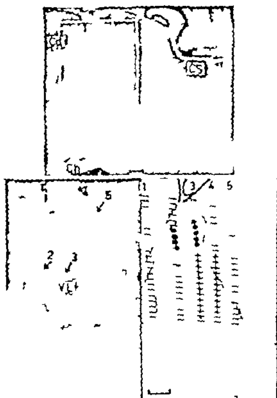


Fig. 8. Sagittal section through the brain stem about 5 mm laterally to the midline (top). The framed area is shown below to the left in higher magnification. Below to the right is diagram of the same area. Cd, nucleus caudatus. CS, colliculus superior. VL, nucleus ventralis lateralis. Ch, chiasma opticum.

grid in the rostrocaudal direction is more difficult to evaluate. The rostral border of the ventrolateral nucleus is obscure. Therefore it is questionable whether track number 2 passes through, or rostrally to, the nucleus. Tracks 3 and 4, however, no doubt fell within the borders of the ventrolateral nucleus. In the experiment illustrated by Fig. 9 B an electrolytic lesion was made at a point where a typical thalamic response of the type with short latency was recorded. The lesion is seen to be centrally placed in the ventrolateral nucleus.

Unfortunately the material available does not allow a detailed localization of the long latency response seen in the dorsal part of the tracks through the ventrolateral nucleus. In Fig. 9 C and D, however, a track is shown with a lesion made at the depth where the response in that track changed from the long latency type to the response with shorter latency. From the figure it is evident that the response with long latency dorsally to the lesion was not derived from the ventrolateral nucleus. The track seems to have passed in its dorsal part through a pool of cells which may belong either to the nucleus ventralis anterior or the nucleus centralis lateralis.

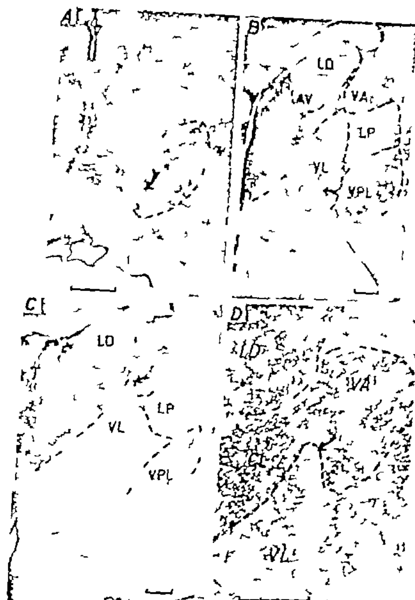


Fig. 9. A. Frontal section through the mesencephalon. The red nucleus is seen within the broken line. An electrolytic lesion is pointed at by the arrow.

B. Frontal section through the thalamus. An electrolytic lesion in the ventrolateral nucleus is seen at the arrow.

C and D. Different magnifications of a section through the thalamus. An electrolytic lesion (arrow) is situated at the dorsal border of the ventrolateral nucleus. Response of the long latency type was seen within about 2 mm dorsally to the lesion.

AV, nucleus anterior ventralis. CL, nucleus centralis lateralis. LD, nucleus lateralis dorsalis. LP, nucleus lateralis posterior. VA, nucleus ventralis anterior. VL, nucleus ventralis lateralis. VPL, nucleus ventralis posterolateralis. Scale 1 mm.

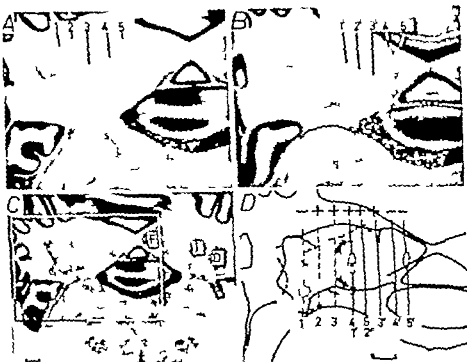


Fig. 10 Frontal section showing stimulating electrode tracks through the cerebellar nuclei. I A track 1 passes down into the dentate nucleus while the other tracks traverse the interposite nucleus. I B tracks 1', 2' and 3' pass through the interposite nucleus while tracks 4' and 5' enter the fastigial nucleus. C is low power magnification of the same section as A. D is composite diagram of A (nuclear borders drawn in broken lines) and B (nuclear borders drawn in full). Plus symbols indicate tracks in which electrical stimulation evoked response in the red nucleus and in the ventrolateral nucleus. F nucleus fastigii I, nucleus interpositus. D nucleus dentatus Scale 1 mm.

III *Localization of stimulating points*

These experiments were aimed at localizing the area within the cerebellar nuclei which, when electrically stimulated, activated the cells in the red nucleus and in the ventrolateral nucleus of the thalamus. A microelectrode was kept stationary either in the red nucleus or in the ventrolateral nucleus. The cerebellar nuclei were then investigated with mediolateral or rostrocaudal rows of stimulating electrode tracks. Fig 10 and 11 are illustrations of such experiments. Fig 10 C shows a low power magnification of a histological section through the cerebellar nuclei. A higher magnification of the framed area is seen in Fig 10 A. Tracks 1 2, 3 4 and 5 are passing through the lateral part of the cerebellar nuclear complex. A lesion made by electrocoagulation is seen at the bottom of track 1 It is placed in the dentate nucleus In Fig 10 B is shown a similar section from another experiment where tracks 1' 2' 3' 4' and 5' enter the medial part of the nuclear complex. Electrocoagulations



Fig. 11 Sagittal section through the cerebellum about 4 mm laterally to the midline. The interposite nucleus with five stimulating electrode tracks is seen. Below is diagram of the same section. Thick lines along the tracks indicate part of tracks effective in evoking a response in the red nucleus. The center of the electrocoagulation was used as reference when measuring in the thick lines. Scale 1 mm.

are seen placed in the interposite (track 1) and the fastigial (track 5) nuclei. Fig 10 D is a composite diagram of A (borders of the nuclei and tracks drawn *in full) and B (borders and tracks drawn in broken lines) Electrical stimulation* in the tracks with plus symbols above gave responses in the red nucleus and in the *ventrolateral* nucleus. *In the dorsoventral direction stimulation* was effective within the arrows in track 4/1 This distance was determined with the center of the electrocoagulation (cross) in track 1 as a reference. *It appears from Fig 10 that only tracks passing through the interposite nucleus* evoked a response in the red nucleus and in the investigated part of the thalamus. Tracks through the dentate and the fastigial nuclei were always negative in this respect. These observations were confirmed without exception in all experiments. An interesting observation was made in the experiments of the type illustrated in Fig 11 *In these rostrocaudal rows of tracks it was* always noticed that the more caudally the stimulating electrode was situated within the interposite nucleus, the higher the stimulating intensity had to be in order to give a response of a certain amplitude In fact, stimuli applied to the most caudal parts of the nucleus were rather ineffective. *It is also evident* from Fig 11 that stimulation *of the brachium conjunctivum rostrally and* ventrally to the cerebellar nuclei was effective in evoking the *focal potentials* in the red nucleus and in the ventrolateral nucleus.

Discussion

The results presented seem to prove that stimulation of the interposite nucleus in the cerebellum activates the cells in the red nucleus and in the ventrolateral nucleus of the thalamus. This is in agreement with the findings of JANSEN and JANSEN (1955) that the bulk of the fibres from the interposite nucleus end in the red nucleus. This view was also supported by POMPEIANO (1958) who showed that motor effects elicited by electrical stimulation of the cerebellar cortex were abolished by destruction of the interposite nucleus or the red nucleus.

JANSEN and JANSEN also found that some fibres from the interposite nucleus, together with the majority of the dentate fibres, passed beyond the red nucleus. These fibres were thought to have thalamic connections. The present investigation has proved this view to be true with regard to the interposite fibres. On the other hand, it failed to show any connections between the dentate nucleus and the thalamus. From JANSEN and BRODAL (1958) it seems evident, however that experiments directly demonstrating a relationship between the ventrolateral nucleus of the thalamus and the dentate nucleus are still lacking. The present findings do not support the view that such a relationship exists. It can, however not be excluded that the dentate nucleus may have connections in other thalamic nuclei. Nor can it be excluded that the dentate nucleus impinges upon the cells in the ventrolateral nucleus (and maybe also in the red nucleus) in a way which is not elucidated by the technique used in this investigation.

An interesting detail in the work by POMPEIANO was that the lesion in the interposite nucleus had to be situated in the rostromedial part of the nucleus in order to abolish the motor effects observed. Experiments of the type shown in Fig. 11 in the present investigation seem to support this observation in that electrical stimulation is more effectively evoking the rubral response if carried out rostrally in the interposite nucleus.

The distance between the interposite nucleus and the red nucleus as measured on a histological section (shrinkage accounted for) is approximately 9 mm. The mean latency to the maximum of the initial positivity in the presynaptic spike recorded in the red nucleus is 0.49 msec. Adopting the interpretation of BROOKS and ECCLES (1947) this point signals the arrival of the presynaptic volley to the red nucleus. The conduction velocity may therefore be estimated to be about 18 m/sec. In the ventrolateral nucleus the corresponding latency is 0.94 msec and the total distance between the cerebellum and the thalamus is about 17 mm. The conduction velocity computed from these figures is also about 18 m/sec. As the difference in latency between the thalamic response and that recorded from the red nucleus is only 0.4 msec there seems to be no time for a synaptic delay in the path. The uniform conduction velocity computed from measurements made both caudally and rostrally to [illegible] nucleus also suggests that the path from the cerebellum to the [illegible] a direct one and not interrupted by a synapse in the red nucl[illegible]

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Oxygen Consumption and Sodium Reabsorption in the Kidney

By

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Abstract

LASSEN, N. A., O. MUNCK and J. H. THAYSEN. *Oxygen consumption and sodium reabsorption in the kidney.* Acta physiol. scand. 1961. *51.* 371—384. — Renal oxygen consumption can be divided into a small but constant basal oxygen uptake and a variable suprabasal or functional oxygen uptake. The basal oxygen consumption is defined as the oxygen uptake of the non-filtering and hence non-reabsorbing kidney. It was measured during marked hypotension and was found to amount to 1 µmole of O_2 per gram kidney per minute. The suprabasal or functional oxygen consumption is defined as the total oxygen uptake minus the basal. It was measured on normally functioning kidneys and was found to correlate very closely with the quantity of sodium reabsorbed. For each mol of oxygen consumed in excess of basal requirements 28 sodium equivalents were reabsorbed. The hypothesis, that sodium reabsorption represents the main renal work is forwarded. It provides a simple explanation for the various classical observations on renal oxygen uptake and is in agreement with data on other electrolyte transporting tissues.

In the isolated frog skin it was found by ZERAHN (1956 and 1958) that there was a basal or resting oxygen consumption, when the medium on the outside of the skin was sodium-free. When sodium was added to the medium, the skin started to transport sodium, and a functional oxygen uptake was now superimposed on the basal. This suprabasal or net oxygen uptake was dominated by and proportional to active sodium transport.

There is good evidence that about 99 per cent of the filtered volume in the mammalian kidney is reabsorbed in the tubules by a primary active inward transport of sodium, anions and water following passively due to the electrostatic and osmotic forces thus created. On a molar basis sodium reabsorption represents the overwhelming fraction of all tubular transport processes. Therefore, following the suggestion by USSING *et al.* in a recent review (1960) the present study was undertaken with the aim of investigating, whether a correlation between sodium reabsorption and oxygen uptake could also be demonstrated in the intact mammalian kidney. The results, which have been previously communicated briefly by THAYSEN, LASSEN and MUNCK (1959, 1960) are here presented and discussed in full detail.

Methods

Two different types of experiments were included in the study.

A. Measurement of the oxygen uptake of the non-filtering kidney

Four mongrel dogs were used. They were anesthetized with nembutal and throughout the experiment oxygen was administered at a rate of 1—2 l per min through a tracheal tube. The animals were heparinized. The abdomen was opened through a median incision and suitably large catheters tied into both ureters, one femoral artery and the right renal vein. Renal blood flow was measured by the free outflow method and the mean arterial blood pressure determined with a mercury manometer. Blood pressure was reduced to about half of its initial value by bleeding. When renal blood flow had been observed to remain stable for about 5 min samples of arterial and renal venous blood were drawn for determination of oxygen and plasma inulin. The stability of the renal blood flow was controlled after removal of the blood samples. By repeated bleedings blood pressure and renal blood flow were reduced stepwise. At each level the observations were repeated.

B. Measurement of the oxygen uptake and sodium reabsorption of the filtering kidney

Ten mongrel dogs were used. The preparation of the animals was the same as described above except that sampling of renal venous blood in these non-heparinized dogs was carried out with a thin catheter introduced from the external jugular vein and guided manually into the left renal vein. A priming injection followed by a sustained infusion of paraaminohippurate (PAH) and inulin in isotonic saline was commenced in order to maintain arterial plasma concentrations of PAH and inulin of about 5—8 and 50 mg/100 ml, respectively. In exp. no. 9 and 10, urea was added to the infusate in order to secure an adequate urine flow. Two to three hours elapsed between the start of the infusion and the beginning of the measurements. In dogs no. 1 to 8 no attempts were made to interfere with renal blood flow. In dogs no. 9 and 10 the renal venous pressure was maintained at about 30—40 mm Hg by constriction of the renal vein throughout the study. 5—7 observation periods, each of 20—30 min duration, were obtained per dog. Diuresis was measured and the urine analyzed for PAH, inulin and sodium. In the middle of each period blood was drawn simultaneously from the femoral artery and the renal vein and examined for hematocrit, oxygen content, oxygen saturation, plasma inulin, plasma PAH and plasma sodium.

The correct position of the catheters was controlled at autopsy and the kidney

Table I. The basal oxygen consumption of the intact canine kidney

Dog	Observation period no.	Mean arterial blood pressure	Blood flow (ml/g/min)	(A—R) O (µmoles/ml)	Renal oxygen uptake (µmoles/g/min)
A	1	58	1.44	0.72	1.04
	2	45	0.86	0.99	0.85
	3	30	0.35	2.29	0.80
B	1	65	1.06	1.04	1.10
	2	67	1.03	0.78	0.80
	3	60	1.06	1.03	1.09
	4	39	0.53	1.82	0.96
C	1	52	1.06	1.09	1.16
	2	37	0.57	1.64	0.93
D	1	47	0.62	1.81	1.12
	2	45	0.38	2.11	0.80
	3	44	0.52	1.63	0.85

Mean = 0.96 ≃ 1.0
S. E. of mean = 0.04

were weighed after removal of capsule, pelvic fat and large vessels. In exp. 9 and 10 the experimental kidney was somewhat swollen, and the weight of the other kidney was used for the calculations.

Analytical methods and calculations

Blood oxygen content was measured manometrically as indicated by VAN SLYKE and NEILL (1924). The analyses were completed within 30 min after sampling, and the values obtained were compared to the results of simultaneous duplicate, photoelectric measurements of the oxygen saturation. Only the manometric analyses were used in the calculations. Inulin in plasma and urine was measured according to the method of ALVING, RUBIN and MILLER (1939). PAH was determined by the method of BRUN (1951). Sodium was measured by internal standard flame photometry. The hematocrit was measured by centrifugation. No correction was made for plasma trapping.

Renal plasma flow (RPF) in ml/g/min was calculated for each dog as the mean value of the plasma flows in the individual periods. Similar calculations were carried out for the hematocrit (H), the arterial-renal venous oxygen difference (A — R)O$_2$ in µmole/ml, the glomerular filtration rate (GFR) in ml/g/min, the plasma sodium concentration (P_{Na}) in µeq/ml, and for the urinary excretion rate of sodium (U_{Na} × V) in µeq/g/min.

From these values the following parameters were calculated

$$\text{Filtration fraction (FF)} = \frac{GFR}{RPF}$$

$$\text{Sodium reabsorption (Na reabs.)} = GFR \times P_{Na} - U_{Na} \times V \ (\mu eq/g/min)$$

$$\text{Total renal oxygen uptake } (QO_2\text{total}) = \frac{RPF}{1-H} \times (A-R)O_2 \ (\mu mole/g/min)$$

Table II. Studies of renal sodium transport and oxygen metabolism in ten dogs. In dogs no. 1—8 no attempt was made to interfere with blood flow. In no. 9 and 10 the renal venous pressure was elevated to 50—60 mm Hg

Dog no.	No. of periods	RPF ml/g/min	H	RBF ml/g/min	(A—R) O_2 µmoles/ml	GFR ml/g/min	FF	Plasma sodium µeq/ml	Na filtr µeq/g/min	Na excret. µeq/g/min	Na reabs. µeq/g/min	QO_2 total µmoles/g min	QO_2 net µmoles/g min	Na/net O_2 ratio
1	4	1.89	0.41	3.20	1.26	0.66	0.35	139	91.7	0.4	91.3	4.0	3.0	30
2	7	3.92	0.40	6.53	1.06	1.13	0.29	156	176.3	6.9	172.5	6.9	5.9	29
3	3	3.26	0.51	6.65	1.29	1.28	0.39	153	195.8	3.7	192.1	8.6	7.6	25
4	6	2.63	0.48	5.06	1.03	0.82	0.31	140	114.8	1.3	113.5	5.2	4.2	27
5	6	3.88	0.42	6.69	0.88	0.87	0.22	148	128.8	2.8	126.0	5.9	4.9	26
6	5	3.43	0.54	7.46	0.74	0.88	0.26	140	123.2	0.8	122.4	5.5	4.5	27
7	3	2.33	0.46	4.31	0.90	0.70	0.30	144	100.8	0.2	100.6	3.9	2.9	35
8	3	2.91	0.23	3.78	1.21	0.66	0.23	159	104.9	1.8	103.1	4.6	3.6	29
9	4	0.42	0.38	0.68	3.02	0.14	0.33	171	23.9	1.2	22.7	2.1	1.1	21
10	4	1.33	0.45	2.46	1.34	0.51	0.38	150	76.5	0.6	75.9	3.3	2.3	33
													Mean	= 28
													S.D.	= 4

Net renal oxygen uptake (QO_2net) = QO_2total — oxygen uptake of the non-filtering kidney

Na/net O_2 ratio = number of sodium equivalents reabsorbed per mole oxygen =

$$= \frac{\text{Na reabs.}}{QO_2 \text{ net}}$$

Experimental accuracy

The coefficient of variation in the determination of average net or suprabasal oxygen uptake was calculated to 13 per cent and that in the determination of sodium reabsorption to 4 per cent. These calculations are based on in average 4.5 observations of each parameter in the individual experiment.

Results

A. *The oxygen uptake of the non-filtering kidney*

Table I gives the values for mean arterial blood pressure, renal blood flow, arterial-renal venous oxygen difference and renal oxygen uptake. As demonstrated graphically in Fig. 1 (part A) the kidney studied during severe hypotension was found to react to a further reduction in blood flow with an increase in the a.v. oxygen difference, whereas the oxygen uptake remained fairly constant. Since no measurable a.v. difference for inulin could be detected in these experiments, we have interpreted the values for renal oxygen uptake as indicating the oxygen uptake of the non filtering kidney. It was found to average 1.0 µmole/g/min.

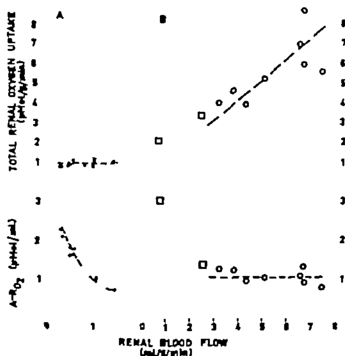


Fig. 1. A. Renal oxygen uptake of the non-filtering kidney in four dogs. Each point represents one observation period.
B. Renal oxygen uptake of the filtering kidney in ten dogs. Each point represents the average of 3—7 observation periods. In the experiments indicated by O no attempt was made to interfere with renal blood flow. In the experiments indicated by □ the renal vein was constricted.

B. *The oxygen uptake of the filtering kidney*

Table II gives the observations made in the dogs with normally functioning kidneys. The data have been analysed in some detail and the results of this analysis are presented in the following paragraphs.

The relationship between blood flow and total oxygen uptake

Fig. 1 (*part B*) *shows that the a.v. oxygen difference remained fairly constant* at about 1 µmole/ml in the 8 dogs with normal renal venous pressure despite variations in blood flow from 3.2 to 7.5 ml/g/min. In these experiments the total renal oxygen uptake consequently showed a rough correlation to renal blood flow. In the two experiments where renal venous pressure was increased, the a.v. oxygen difference was rather high and thus the total oxygen uptake was not reduced quite in proportion to the reduction in blood flow. Hence the observed rough correlation between blood flow and oxygen uptake may be upset by a marked increase in renal venous pressure.

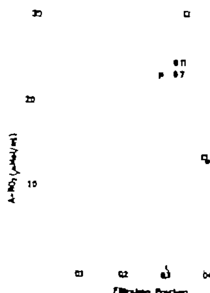


Fig 2. Relationship between renal arteriovenous oxygen difference and filtration fraction.
In the experiments indicated by O no attempt was made to interfere with renal blood flow In the experiments indicated by ▢ the renal vein was constricted.

The relationship between filtration fraction and arteriovenous oxygen difference

Fig 2 shows that no significant correlation existed between these two parameters.

The relationship between filtration rate and total oxygen uptake

There was a highly significant correlation between the filtration rate and the total renal oxygen uptake. As seen in Fig 3 the data suggest a linear relationship, and the calculated regression line indicates that 5 7 μmoles of oxygen are consumed per ml of filtrate formed The intercept c represents the oxygen consumption of the kidney when no f place and was found to be 0.6 μmole/g/min (± 0.4) This lower than the value determined during haemorrhagic hyp difference is not statistically significant.

The relationship between sodium reabsorption and total oxygen uptake

The correlation between these two parameters was equa above described (Fig 3) The coefficient of correlation betw sorption and total oxygen uptake was a little higher (r = 0. filtration rate and total oxygen uptake (r = 0.97) altho is not significant. It is not surprising that these two correlati since, in the present experiments, the filtration rate and sorption were highly intercorrelated due to the small sodium concentration and in the excreted fraction of t

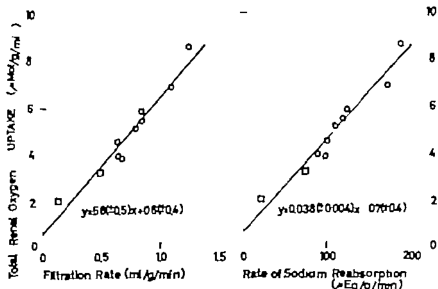


Fig. 3. Relationship between total renal oxygen uptake and filtration rate, and between total oxygen uptake and sodium reabsorption.
In the experiments indicated by O no attempt was made to interfere with renal blood flow
In the experiments indicated by □ the renal vein was constricted.

The ratio between sodium reabsorption and net oxygen uptake

The functional or net oxygen uptake was calculated by subtracting from the total oxygen uptake (in μmoles/g/min) the oxygen uptake of 1 μmole/g/min which was found in the non-filtering kidney The ratio between sodium reabsorption and net oxygen uptake, the Na/net O ratio, was on an average 28 with a standard deviation of 4 i.e this value varied but little from dog to dog. The ratio denotes that 28 sodium equivalents were reabsorbed per mole of oxygen consumed.

Discussion

The basal oxygen uptake of the kidney

The definition of the basal or resting oxygen uptake of the kidney depends upon what we consider to be the major work process of the tubular epithelium, and its determination requires interference with normal physiological conditions, because the kidney is a normally never resting gland. Since the work of the tubular cells is to modify the glomerular filtrate into final urine, mainly by reabsorption of the filtrate, we have defined the basal oxygen uptake of the kidney as the oxygen uptake when no filtration and hence no reabsorption takes place We have determined this basal metabolism in the artificial anu-

Table III. The basal oxygen consumption in various tissues (recalculated to µmoles of O_2 per g wet tissue; for references see Ussing et al. 1960)

Organ	Species	Reference	Basal oxygen uptake (µmoles/g/min)
Submaxillary gland	Dog	Terroux et al. (1958)	1
Submaxillary gland	—	Barcroft (1916)	1.0
Parotid gland	—	Ohara (1951)	1.1
Pancreatic gland	—	Still et al. (1933)	1.2
Kidney	—	Present Study	1.0
Kidney	Man (acute renal failure)	Munck (1958)	1.0

ation of severe hypotension produced by bleeding and a fairly constant value over a wide range of variation in renal blood flow was found. Despite the unphysiological conditions this basal renal oxygen uptake of 1 µmole/g/min comes very close to the oxygen uptake in different non-stimulated exocrine glands and to the oxygen uptake of the anuric human kidney (Table III).

The finding of an intercept of about 0.6 µmole/g/min for the regression of total oxygen consumption on filtration rate or sodium reabsorption further supports the contention, that the kidney has a small, but constant basal oxygen uptake. The basal oxygen consumption obtained from these regressions is of the same order of magnitude as that obtained in the studies of the non-filtering kidney, but it is less exactly determined.

The basal oxygen uptake is probably used for preservation of the cellular structure and for extrusion of sodium leaking into the tubular cells over the inner (blood side) membrane. The small variations in the basal oxygen uptake from experiment to experiment suggest that the sodium leak is constant and relatively small.

The functional or net oxygen uptake of the kidney

As this value was determined by subtracting a constant from the total oxygen uptake, the net oxygen uptake has exactly the same highly significant and linear correlation to filtration rate and sodium reabsorption as the total oxygen uptake. When using the net oxygen uptake values the intercept on the y-axis practically disappears, demonstrating a direct proportionality between net oxygen uptake and filtration rate and between net oxygen uptake and sodium reabsorption. This suggests that either water reabsorption or sodium reabsorption is the dominant oxygen requiring work of the tubular epithelium. No other tubular transport process or synthesizing activity can be expected to parallel the filtered volume to such an extent. Moreover on a molar basis

all such tubular processes are negligible as compared to water and sodium transport. The present experiments do not permit any conclusion as to whether water or sodium reabsorption requires the oxidative energy since the two are highly intercorrelated. However according to modern concepts reabsorption of filtrate takes place by a primary active reabsorption of sodium ions.

The following hypothesis regarding the renal oxidative metabolism is thus formulated. The total renal oxygen uptake comprises a relatively small and constant basal oxygen uptake of 1.0 µmole/g/min and a variable suprabasal or functional uptake which is utilized for sodium reabsorption at a rate of 1/28 mole O_2 per equivalent of Na reabsorbed. In quantitative terms this may be expressed by equation 1 (all values per g kidney per min)

$$QO_2\ \text{total} = (A - R)O_2 \times RBF = QO_2\ \text{basal} + QO_2\ \text{net} = 1.0 + 1/28\ \text{Na reabs} \quad (1)$$

In the next paragraphs this hypothesis will be analyzed on the basis of various observations in the literature regarding renal oxygen consumption.

The proportionality between oxygen uptake and blood flow

In most organs a moderate reduction in blood flow leads to an increase in arteriovenous oxygen difference, so that the oxygen uptake remains unchanged. The kidney does not follow this pattern. It is evident from numerous observations — including the present series — that the renal a.v. oxygen difference most often is relatively independent of blood flow except at very low flows, so that there is rough proportionality between oxygen uptake and blood flow (Hayman and Schmidt (1928), Glaser *et al.* (1932), Van Slyke *et al.* (1934), Levy *et al.* (1937, 1938), Levy and Blalock (1938), Kramer and Winton (1939), Dole *et al.* (1946), Bradley and Halperin (1948), Bucht *et al.* (1949), Carroll and Hickam (1949).)

This peculiar behaviour of renal oxidative metabolism has caused much speculation. It has been suggested that the renal parenchyma was unable to extract oxygen from the blood below a certain critical limit. This theory was rejected by Dole *et al.* (1946), who showed that the a.v. oxygen difference did not change at low arterial oxygen saturations.

The present hypothesis readily explains the relation between blood flow and oxygen uptake, as glomerular filtration and also tubular sodium reabsorption usually decrease in proportion to a decrease in blood flow. Thus the difference between the oxidative metabolism of the kidney and that of other organs is not due to a metabolic peculiarity of renal cells, but it is a mere consequence of the unique functional organization of the kidney. The amount of work to be carried out by the tissue is to a large extent determined by blood flow. When this functional pattern is abolished by marked hypotension stopping the filtration, the kidney — like any other organ — extracts more oxygen per ml of blood in response to a further reduction

Table IV Ratio of active electrolyte transport to net oxygen consumption in various tissues

Tissue	Ratio	Reference
Canine kidney	28	Present Study
Human kidney	30	Calculated from data of CARGILL & HICKAM and CLARK and BARKER
Submaxillary gland.	16—25	Calculated from data of TERROUX, SEKELJ and BURGEN
Isolated frog skin	16—20	ZERAHN

perfusion and maintains a constant oxygen uptake until a very low rate of blood flow is reached (cf. Fig 1)

In contrast with the above cited two groups of investigators have found that the renal oxygen uptake is independent of renal blood flow CRUICKSHANK and TAKEUCHI (1925) observed in cats that an increase in the renal venous pressure produced a decrease in blood flow with an increase in a.v oxygen difference. In experiments on dogs where blood flow was measured with a bubble-flowmeter connected to the renal vein GRUPP *et al* (1958) found that the a.v oxygen difference was dependent on the blood flow In these two reports no data are given for filtration fraction or filtration rate, but the experimental conditions are suggestive of a high filtration fraction, so that also these results may well be in accordance with the concept that oxygen uptake is dependent on sodium reabsorption. This is discussed in more detail in the following.

The narrow arteriovenous difference and its variations

CLAUDE BERNARD (1858) first described the high oxygen contents of renal venous blood, an observation which suggests an ample supply relative to demand. In the terms of the present hypothesis the narrow a.v difference $(A-R)O_2$ is the result of the fact, that only about 20 percent of the plasma flowing to the kidney is filtered under usual conditions that the oxygen cost of sodium reabsorption is only 1/28 mol of O_2 per equivalent of sodium reabsorbed, and that the basal oxygen uptake is sufficiently small as to cause only a negligible oxygen extraction at the normal rate of perfusion.

The relative constancy of $(A-R)O_2$ when blood flow (*RBF*) is being reduced moderately has been commented on already But the present theory even permits to make exact quantitative predictions of $(A-R)O_2$ in any state of function since equation (1) can be solved for $(A-R)O_2$ to yield equation (2) when inserting the previously defined symbols

$$(A-R)O_2 \text{ in } \mu mol/ml = \frac{QO_2 \text{ total}}{RBF} = \frac{1.0}{RBF} + \frac{1}{28}(1-H)FF \times P_{Na}(1-E_{Na}) \quad (2)$$

When RBF is very small then $(A-R)O_2$ will increase quite markedly even when the sodium reabsorption per ml of blood $((1-H)FF \times P_{Na}(1-E_{Na}))$ is unchanged. Our experiment no. 10 illustrates this phenomenon which upsets the gross correlation between QO_2 total and RBF quite markedly. Further equation (2) explains the large $(A-R)O_2$ in incompensated heart disease, since in this condition RBF is low and FF high (MERRILL 1946). The very narrow $(A-R)O_2$ found during albumin infusion also fits into equation (2) since here RBF is high and FF low (BARKER *et al.* 1949). Finally equation (2) permits to predict that no strict correlation can be expected to exist between $(A-R)O_2$ and FF unless all the other variables are kept reasonably constant. This lack of significant correlation between $(A-R)O_2$ and FF was observed by DOLE *et al.* (1946) and also in the present series (cf. Fig. 2). On this basis DOLE *et al.* rejected the possibility that the renal oxygen uptake should be dependent on the amount of filtrate to be handled by the tubular tissue. Their conclusion was probably wrong, as the present data show that there was a highly significant correlation between *net* oxygen uptake and sodium reabsorption even though $(A-R)O_2$ and FF varied independently. DOLE *et al.* did not consider the possibility of a basal oxygen uptake.

Miscellaneous observations on renal oxygen uptake. CARGILL and HICKHAM (1949) and CLARK and BARKER (1951) have published data on the oxygen uptake of the normal human kidney. Their average value from a total of 28 observations can be calculated to be 3.1 µmoles/g/min when assuming a renal weight of 300 g. From their data for the glomerular filtration rates the average sodium reabsorption in the same subjects comes at 60 µeq/g/min assuming a plasma sodium concentration of 142 µeq/ml and a negligible sodium excretion. From these data in man a Na/netO_2 ratio of about 30 is obtained, i.e. almost the same value as in the present experiments on dogs.

BECHT *et al.* (1949) and CLARK and BARKER (1951) studied the effect of variations of renal PAH excretion on renal oxygen uptake in man. They found no significant correlation between the two parameters. This is in agreement with the present hypothesis since the maximal PAH transport measured in eq/min is very small compared to sodium reabsorption. Similarly variations in renal glucose or aminoacid reabsorption cannot be expected to influence renal oxygen uptake measurably. Moreover the possibility of a coupling between sodium transport and one or more of the other transport mechanisms cannot be excluded, a possibility implying that these other transport mechanisms may not *per se* require oxidative energy.

A very interesting observation was made by CARGILL and HICKAM in studies of the renal oxygen uptake in chronic renal diseases. In these diseased kidneys a direct proportionality was found between the reduction in total oxygen

uptake and in glomerular filtration rate. This is in agreement with the present hypothesis if it is assumed that the weight of metabolically active renal tissue and hence basal oxygen uptake was also reduced roughly in proportion to the reduction in glomerular filtration rate.

The renal efficiency The minimal energy required to form urine from plasma ultrafiltrate can be calculated by applying simple thermodynamic considerations to the quantitatively important substances (VON RHORER 1901). This minimal energy constitutes only about 1 per cent of the total energy released by the oxidative metabolism in the kidney (BORSOOK and WINEGARDEN 1931). This value of about one per cent for the efficiency of renal work is surprisingly low compared to that of the muscles and moreover it was shown to vary considerably (EGGLETON, PAPPENHEIMER and WINTON 1940) In view of the present hypothesis thermodynamic calculations of the minimal renal work are irrelevant since they disregard the utilization of oxygen for active sodium reabsorption. Since the present theory of renal work is based on the stoechiometric considerations formulated by ZERAHN for the frog skin the concept of a renal efficiency in the usual thermodynamic sense of the word cannot be used.

The hypothesis that the net (suprabasal) oxygen consumption of the kidney is dominated by and stoechiometrically related to active tubular sodium reabsorption has been shown to be consistent with a series of classical observations on the oxidative metabolism of the kidney It is of interest in this context that the oxygen consumption of exocrine glands other than the kidney is apparently also best explained in terms of active electrolyte transport (USSING *et al.* 1960)

When not secreting the exocrine glands have a relatively constant basal oxygen uptake (Table III) When stimulated to discharge a functional oxygen consumption is superimposed on the basal. The suprabasal or functional oxygen consumption must be largely used for the processes underlying glandular discharge, since the synthesis of enzymes and other specific substances proceeds at a relatively constant rate under all conditions. In the glands as in the kidney the functional oxygen consumption has been correlated with the thermodynamic work involved in the formation of a secretory product deviating in ionic composition from the original plasma. A minimal "glandular efficiency" was found in this way

Such calculations, however disregard the oxygen cost of the isotonic active outward transport of electrolyte (water following passively) which represents the basis of the secretory process according to modern concepts of glandular function. If one calculates from data in the literature the ratio between active electrolyte transport and net oxygen consumption this ratio, the stoechiometric efficiency is found to vary from 16 to 25 (Table IV)

In 1958 TERROUX *et al.* showed that the arteriovenous oxygen difference in the non-stimulated salivary gland was 3—4 μmol/ml. When the gland was stimulated to secrete the a. oxygen difference decreased considerably and

approached that found in the kidney With the knowledge that glandular blood flow varies roughly in proportion to secretory rate and that the secretion fraction[17] like the filtration fraction of the kidneys, is about 0.25 the observations of TERROUX et al can be explained on the basis of the concepts developed in the present paper At high rates of blood perfusion the basal oxygen demands of the gland and of the kidney only contribute insignificantly to the a.v oxygen difference. In both gland and kidney net oxygen demands dominate the a.v oxygen difference in this situation. Net oxygen demand per ml of perfusing blood is determined by active outward (gland) or inward (kidney) transport of comparabl quantities of electrolytes originating from secretion or filtration fractions of the same order of magnitude.

The ratio between active electrolyte transport and net oxygen consumption of the kidney is compared to the ratios found in other intact organs and in the isolated frog skin in Table IV Whether the difference between the epithelial membranes (isolated or intact) are significant is difficult to state, and depends to some extent on the accuracy with which the basal oxygen consumption of the membranes is determined The larger the basal uptake is in comparison to total uptake during maximal transport, the larger error may arise from an inaccurate determination of basal metabolism. For these reasons it is as yet impossible to stat whether the stoechiometric efficiency of the electrolyte transporting mechanism aries from one epithelial membrane to the other

Addendum in proof Recently ULRIK LASSEN and J H. THAYSEN (Biochim. et Biophys. Acta. 47 616, 1961) demonstrated an intimate correlation between the active sodium extrusion from and the suprabasal oxygen consumption of renal cortical slices from rabbits. A Na/net O ratio of about 25 was found. KRAMER and DEETJEN (Excerpta Medica, Congr Ser. 29-50, 1960; Klin. Wschr 38 680; 1960 Arch. ges. Physiol. 271 782, 1960) have obtained similar results on the intact canine kidney as those presented in the present paper

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The Contribution of the Distribution Factor to the A a Po_2 Difference (A Correction)

By

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In a recent publication on the alveolo-arterial gas exchange (1960) we found that the arterial oxygen deficit in rest breathing air was 0.36 vol of which the part due to true venous admixture was found to be 0.08 vol / Since the part of the *A—a Po* difference due to incomplete diffusion equilibrium can be considered to be negligible the remainder of the arterial oxygen deficit 0.36 vol —0.08 vol / = 0.28 vol in air will be due to the distribution factor

The effect of the distribution factor on the *A—a Po* difference must at different levels of alveolar Po_2 be proportional to the change of slope of the O_2-dissociation curve, to the second derivative of this curve. The contribution of the distribution factor to the arterial oxygen deficit in the experiments with 12 % O was, therefore, estimated t be about 20 times larger than during air breathing *i. e* sufficient to cover the whole arterial *O* deficit at 12 O_2. By this estimation we unfortunately overlooked the fact that the same difference in ventilation/perfusion ratio produces a smaller *Po* difference between *th relatively hypo- and hyperventilated parts of the lung* at 12 O than t 21 O_2. This is caused by the fact that, due to the shape of the O -dissociation curve, a given relative hypo entilation will d minish the oxygen uptake in the hypoventilated part of the l ng more at 12 O than at 21 O_2. Correspondingly the oxygen uptak n the hyperventilated part of the lung will ncrease more at 12 O than at 21 O_2.

W have calculated the alveolar Po_2 and Pco_2 differences between *relatively* hypoventilated and hyperventilated parts of the lung at 21 O and at 12 O in two cases of uneven distribution. It was *assumed that the ventilation in the* hypoventilated part of the lung was reduced to 50 or to 67 of the mean,

and, in the hyperventilated part, increased to 150 % or 133 % while the circulation remained constant. It was tentatively assumed that the alveolar gas tensions were equal to the end pulmonary capillary gas tensions. Further it was presumed that no regulatory changes in ventilation and circulation took place at 12 % O The respective alveolar P_{CO_2} and P_{O_2} values were determined as those that allowed the gas exchange from the inspired air to the alveoli to equal the gas exchange from the alveoli to the lung capillaries in each of the two parts of the lung while at the same time the total gas exchange remained constant. The calculations showed that the P_{O_2} difference between the hypoventilated and the hyperventilated parts of the lung in both cases was 3.5 to 4 times smaller at 12 % O than at 21 % O_2.

The decrease in O saturation, ΔS % of the arterial blood that occurs when equal amounts of blood from the relatively hypoventilated and the relatively hyperventilated parts of the lung are mixed can be expressed as

$$\Delta S\ \% = \tfrac{1}{8}(\Delta P)\ + \left(\frac{d^2S}{d\,P^2}\right)P$$

where ΔP is the difference in alveolar P_{O_2} between the two parts of the lung and $\left(\frac{d^2S}{d\,P}\right)P$ i the second derivative of the O dissociation curve at mean alveolar P_{O_2}.

Since ΔP was found to be 3.5 to 4 times smaller at 12 O than at 21 % O whereas the second derivative of the oxygen dissociation curve (curve III Fig 3 ASMUSSEN and NIELSEN 1960) is 22 times larger it follows that at 12 % $O_2 \Delta S$ % is only about 1 5 times larger than at 21 % O_2. As the part of the arterial O deficit which was due to the distribution factor was found to be 0.28 vol at rest breathing air this factor will increase to about 0.42 vol at 12 % O_2. True venous admixture (0 08 vol) and uneven distribution (0 42 vol) will, therefore, contribute 0 5 vol % or 25 of the total arterial deficit which at 12 O was found to be 2 vol %. The remaining part (75 %) of the arterial O deficit must be assumed to be due to failing diffusion. The lung diffusion constants for O calculated under the *erroneous* assumption that at 12 % O the main part of the arterial oxygen deficit was due to uneven distribution are consequently too high

By the calculation it was tentatively *assumed that there was no diffusion* gradient from alveolar air to end capillary blood. The result of the calculation however showed that a diffusion gradient actually exists at 12 % O_2. A precise calculation of the contributions of failing diffusion and uneven distribution to the A—a P_O difference at low oxygen can, therefore, hardly be performed.

Reference

ASMUSSEN, E. and M. NIELSEN Alveolo-arterial gas exchange at rest and during work at different O_2 tensions. *Acta physiol. scand.* 1960. *50* 153—166.

VOL. 51 INDEX

Fasc. 1 (January 1961)

Fasc. 2—3. (February—March 1961)

Fasc. 4 (April 1961)

INDEX AUCTORUM